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REVIEW OF GOVERNMENT SUPPORTED RESEARCH ON AGING

VOLUME II

APPENDIX B - PART I



APPENDIX B - PART I



NICHD RESEARCH GRANTS AND PROJECTS

NICHD RESEARCH GRANTS, FISCAL YEAR 1967

<u>GRANT NUMBER</u>	<u>INVESTIGATOR, PROJECT TITLE</u>
HD 00041	Robert Getty Iowa State University of Science and Technology Ames, Iowa Gerontological Studies in the Dog and Hog
HD 00042	David Brandes Baltimore City Hospitals Baltimore, Maryland Study of Senescence at the Cell Level
HD 00086 HD 00490	Morris H. Ross Institute for Cancer Research Newark, Delaware Effect of Changes in Enzyme Levels on Life Span Aging of Cells and Tissues - Enzymatic Correlations
HD 00146	Gerhard K. Brand University of Minnesota Minneapolis, Minnesota Studies on Aging in Skin
HD 00261	Morris Rockstein University of Miami Coral Gables, Florida Some Physiological Aspects of Aging
HD 00340	George A. Talland Harvard University Boston, Massachusetts Aging and the Selection of Informa- tion
HD 00364	Morton Lieberman University of Chicago Chicago, Illinois Adaptation and Survival Under Stress in the Aged

HD 00407	Malcolm D. Jones University of California San Francisco, California Radiographic and Pathologic Studies of Aging in the Primate Spine
HD 00494	Anita Zorzoli Vassar College Poughkeepsie, New York Age and Cellular Metabolism
HD 00510	Jack Wickstrom Tulane University New Orleans, Louisiana Responses of Bone to Changes in Age and Function
HD 00517	Thomas Jones Angell Memorial Hospital Boston, Massachusetts Disease in Animals Related to Aging
HD 00534	Roy Walford University of California Los Angeles, California The Role of Immune Phenomena of Aging
HD 00571	Morris Rockstein University of Miami Coral Gables, Florida Aging in Musca Domestica L
HD 00599	Jerome Tobis Montefiore Hospital and Medical Center New York, New York Study of Physical Performance of the Elderly Patient
HD 00636	Larry Ewing Oklahoma State University of Agriculture and Applied Science Stillwater, Oklahoma Effect of Aging and Stress on Testicular Metabolism
HD 00642	William B. Weil, Jr. University of Florida Gainesville, Florida Early Nutrition, Body Composition, and Aging

- HD 00643                      Clyde L. Randall  
State University of New York  
Buffalo, New York  
Oophorectomy with Hysterectomy
- HD 00648                      Kurt A. Weiss  
University of Oklahoma  
Oklahoma City, Oklahoma  
Tissue Changes with Aging in  
Highly Inbred Rats
- HD 00668                      Ewald W. Busse  
Duke University  
Durham, North Carolina  
An Integrated Investigation of  
Aging and the Aged
- S.I. Cohen, B.M. Shmavonian, P.L. Hein: Central and Auto-  
nomic Nervous System Mechanisms in Psycho-  
Physiological Responses in Young and Aged  
Persons
- Carl Eisdorfer: Verbal Learning in the Aged: Psychologi-  
cal and Psychophysiological Approaches
- George L. Maddox: Studies on Social Aspects of Aging and  
Human Development
- Walter Obrist: Aging and Cerebral Evoked Responses
- Larry W. Thompson: Hyperbaric Oxygenation, Behavior and  
EEG in the Aged
- HD 00669                      Robert Kohn  
Case Western Reserve University  
Cleveland, Ohio  
An Interdisciplinary Program of  
Research in Aging
- Howard Bensusen: Aging of Collagen
- A. Burnett: Growth, Cell Differentiation, and Aging in Hydra
- J. Edwards: Cell Death in the Insect Nervous System
- H. Houser: Study of the Role of Inadequate Protein Intake  
on the Development and Course of Chronic Illness



Sidney Katz: Multidisciplinary Studies of Illness in Aged  
Persons

LeRoy Klein: Study of Skeletal Collagen Metabolism

Robert Kohn: Aging Studies

Aaron Leash: Aging Colony of Rats

Richard Levy: Relation of Age-Related Changes in Thyroid  
Hormone Metabolism to Gonadal Function

Jerome Liebman: Effect of Restricted Neonatal Growths  
on Atherosclerosis in the White  
Carneau Pigeon

Howard Schneiderman: Biological Action of Juvenile  
Hormone - Maturation and Aging  
in Insects

K. Svec: Occurrence of Nuclear Reactive Factors in Sera  
of Non-Rheumatic Aged Persons

HD 00670

Gordon Ring  
University of Miami  
Coral Gables, Florida  
A Center for the Study of Cellular  
Aging

L.S. Dietrich: Coenzyme Metabolism in Aged and Stressed  
Animals

Bennett Sallman: Cellular Metabolic Changes in the Rat  
Heart with Aging

George E. Schaiberger: Age-Associated Changes in Nucleic  
Acids and Proteins of E. Coli

W. van Wagtenonk: Aging in Paramecium

HD 00672

Murray J. Steele  
New York University  
New York, New York  
Multidisciplinary Study of Aging

Stanley Deutsch: Electrophysiologic Studies of Properties of  
Cell Membranes

Herbert Gershberg: Growth Hormones: Parathyroid Relationships  
in Man as Measured by Response to Acute  
Hypocalcemia

Gerson Lesser and J. Murray Steele: Body Composition in Man;  
Aging and Obesity

William Perl: Biophysical Studies

Joseph Post and Joseph Hoffman: Effects of Age Upon DNA  
Replication

Maxwell Schubert: Changes in Chemical Constitution of  
Cartilage with Age

HD 00674

Quentin Deming  
Yeshiva University  
New York, New York  
Comprehensive Program of Research  
in Aging

I.M. Arias: Hepatic Excretory Function: Its Development  
and Senescence

Peter Barland: Studies of Articular Tissues

Paul Gallop: A Systematic Analysis of the Structure and  
Biosynthesis of Collagen

Jack Geller: Pharmacologic and Endocrine Control of Growth  
of the Prostate

Phillip Henneman: Osteoporosis and the Measurement of Growth  
Hormone in the Plasma

Martin Liebowitz: Degenerative Renal Disease

Paul Royce: Control of Organ Growth; Hypertrophy and Hyper-  
plasia of Kidney

M.H. Williams, Jr.: Degenerative Pulmonary Disease of Man

HD 00703

Robert S. Chang  
Harvard University  
Boston, Massachusetts  
Human Cells in vitro

HD 00769

Freddy Homburger  
Bio-Research Institute, Inc.  
Cambridge, Massachusetts  
Aging Studies in the Syrian Hamster

HD 00977

Denham Harman  
University of Nebraska  
Omaha, Nebraska  
Effect of Antioxidants on the Life  
Span of LAF 1 Mice

HD 00988	David Gottlieb University of Illinois Urbana, Illinois Enzyme Activities in the Development of Fungi
HD 01066	Gilbert B. Snyder University of Miami Coral Gables, Florida Molecular Interactions in Skin
HD 01179	Warren Andrew Indiana University Bloomington, Indiana Age Changes in Cell Populations of Connective Tissues
HD 01186	Earl B. Scott University of South Dakota Vermillion, South Dakota Electron Microscope Studies of Aging
HD 01274	Wilma Donahue University of Michigan Ann Arbor, Michigan Milieu Treatment of Older Mental Patients
HD 01325	Jack Botwinick Duke University Durham, North Carolina Psychophysiological Aspects of Aging
HD 01491	Constantinos Miras University of Athens Athens, Greece Aging and Alpha-Hydroxy Acid Decarbox- ylase of Brain
HD 01502	Theodore T. Tsaltas Jefferson Medical College of Phila- delphia Philadelphia, Pennsylvania Effects of Aging and Hormones on the Cartilage Matrix
HD 01550	Sidney Goldstein Brown University Providence, Rhode Island Changing Consumption Patterns of the Aged

HD 01615	Lissy F. Jarvik New York State Department of Mental Hygiene New York, New York Behavioral and Chromosomal Changes of Aging
HD 01691	Stephen Griew University of Otago Dunedin, New Zealand Aging and the Development of Anticipatory Responses
HD 01833	Bryan P. Glass Oklahoma State University of Agriculture and Applied Science Stillwater, Oklahoma Lens Weight as an Age Determiner in Freetailed Bats
HD 01971	Bennett Sallman University of Miami Coral Gables, Florida Studies in the Aging of Cellular Components
HD 01989	Joseph F. Brinley St. Louis University St. Louis, Missouri Sets in the Speeded Performances of the Elderly
HD 02033	Neil C. Tappen University of Wisconsin Milwaukee, Wisconsin Structure and Function of Bone in Growth and Aging
HD 02119	William J. Felts University of Minnesota Minneapolis, Minnesota Characteristics of Dental-Skeletal Aging
HD 02191	Jon J. Kabara University of Detroit Detroit, Michigan Drugs and Brain Cholesterol During Development and Aging

HD 02194	Robert E. Anderson University of New Mexico Albuquerque, New Mexico The Pathology of Accelerated Aging in Germfree Mice
HD 02207	Jean T. Snook Cornell University Ithaca, New York Diet and Pancreatic Enzymes in Development and Aging
HD 02217	Marie M. Jenkins Madison College Harrisonburg, Virginia Relationships in Aging and Repro- duction in Planarians
HD 02250	James Inglis Temple University Philadelphia, Pennsylvania Age and Short Term Memory
HD 02261	Ralph F. Strebel New York Medical College New York, New York Mechanisms of Tissue Calcification in Aging
HD 02304	Charles Taylor Pennsylvania State University University Park, Pennsylvania Behavioral Components of Attitudes Toward the Aging
HD 02377	Marott F. Sinex Boston University Boston, Massachusetts A Biophysical Study of Fluorescence in Aging Tissue
HD 02416	Mary Adams Case Western Reserve University Cleveland, Ohio Exploration of Illness Crisis in Family of the Aged
HE 02513	Richard U. Hausknecht Mt. Sinai Hospital New York, New York Estrogen Precursors in the Post- Menopausal Female

HD 02558	Moe Bergman Hunter College New York, New York Hearing and Aging: Description and Implications
HD 02566	Joseph T. Wachsman University of Illinois Urbana, Illinois Unbalanced Growth: A Molecular Basis of Death
HD 02582	Daniel Peak Menninger Foundation Topeka, Kansas Effects of Aging on Short-Term Memory
HD 02586	Ernst Simonson Mt. Sinai Hospital Minneapolis, Minnesota Physiology and Pathology of Fatigue
HD 02612	Hans Selye University of Montreal Montreal, Canada Studies of Tissue Development and Aging
HD 02646	Karl Meyer Yeshiva University New York, New York Mucopolysaccharides of Connective Tissues
HD 02718	William Forbes Harvard University Cambridge, Massachusetts The Effects of Aging on Muscular Work in Man
HD 02721	Vincent J. Cristofalo Wistar Institute of Anatomy and Biology Philadelphia, Pennsylvania Carbohydrate Metabolism and Aging <u>in vitro</u>
HD 02741	Albert Lansing University of Pittsburgh Pittsburgh, Pennsylvania Experimental Cytology of Aging Cells and Tissues

HD 02888

Marian E. Swendseid  
University of California  
Los Angeles, California  
Single Amino Acid Deficiencies in  
Aging

HD 02919

Hugh A. Lindsay  
West Virginia University  
Morgantown, West Virginia  
Age and Atrophy of Disuse

HD 00041  
Robert Getty  
Iowa State University of Science  
and Technology  
Ames, Iowa

### Gerontological Studies in the Dog and Hog

This is a systematic and detailed morphological study of changes occurring from birth to senility in the "normal" dog and hog.

Macroscopic and microscopic studies related to aging are conducted on samples collected from hogs (1 day to 8½ years of age) and dogs (birth to 19 years of age) of known genetic history and known diets (both control and experimental). A pure-bred Beagle colony has been in existence in the Department of Veterinary Anatomy since 1958, and the hogs are procured from the Department of Animal Science.

Emphasis to date in interpreting the collected samples is on the cardiovascular system and the nervous system. Studies are also ongoing in the endocrine system, e.g., thyroid, ovary, and adrenals, and in the GI tract, e.g., gastric mucosa. The procedures for this study include histological and histochemical evaluation of all tissue samples, correlation of observed changes of each organ with age, and establishment of prediction equations for growth and senility.

Finally, tables and graphs are being prepared depicting the "normal" aging of the various endocrine organs, cardiovascular system and nervous system under consideration in the dog and hog. Data thus far from histochemical and electron microscope studies demonstrate the presence of lipofuscin pigments in lumbar spinal cord, dorsal root ganglia, paravertebral ganglia, and certain cranial nuclei in the dog and hog.

The presence of spontaneously occurring atherosclerotic plaques in many major blood vessels of the aging hog including coronary arteries, cerebral arteries, thoracic and abdominal aorta, have been observed. Studies of the major blood vessels in the dog revealed morphological findings of athero-, arterio-, and intimal-sclerosis.

Alterations in normal cytoarchitecture of ocular tissues occurring with age are also being studied, utilizing histological techniques and light- and fluorescent-microscopy. Special emphasis is placed on changes in vascularization of the retina, ciliary body and iris. With senescence studies on age-related deposition of pigments in the eye are also being pursued.



Cholesterol analysis of porcine and canine serum from animals on normal and specific dietary regimens, sacrificed at various ages, is also being studied.

Marked histomorphological alterations have been observed in the first year of life in adrenal glands from beagles. The capsule, as well as the trabeculae, cortiomedullary border, and supporting elements in both cortex and medulla, become more collagenous. Fatty metaplasia is observed in increasing amounts in the zona reticularis with advancing age, and in specimens 4 years and older, fatty degeneration is common.

The concentration of lipofuscin granules in the canine myocardium is directly proportional to age, initially appearing at puberty. Studies relating nuclear concentration to fiber size and connective tissue components of the myocardium are also ongoing.

This study will eventually chronicle normal changes which take place in the organism from birth to senility, and will then be a basis for comparison to occurrences of pathologic and physiologic change with aging.

HD 00042  
David Brandes  
Baltimore City Hospitals  
Baltimore, Maryland

#### Study of Senescence at the Cell Level

In many tissues, primarily in the endocrine system, a close correlation has been demonstrated between fine structural organization and chemical properties of cells, and their specific functions. It is also well known that important changes associated with aging occur in functional activity of organ systems.

The accumulation of lipofuscin pigment, i.e., "age pigment," in many organs, such as brain, heart, kidney and liver appears to represent one of the most widely accepted cytological changes correlated with aging. Moreover, a quantitative relationship may exist between chronological age and amounts of age pigment in human cardiac muscle.

Ultrastructure and cytochemistry of cells subsequent to hormonal excess or absence are correlated with aged cells in this study.

Prostate gland and seminal vesicles of estrogen-treated rats are utilized, as well as hypophysectomized and castrated rats. Also, thyroid glands from goitrogen-treated and hypophysectomized rats will be analyzed, along with human preparations from subjects of various ages.

Since electron microscopy and more sophisticated cytochemical techniques have evolved, a relationship has become strikingly evident between lipofuscin pigment and lysosomes. Lysosomes and their contained enzymatic hydrolases play an important role in normal physiological function, e.g., digestion of foreign materials engulfed by phagocytosis and pinocytosis, and autolytic catalysis of the cell's own constituents. They may also be involved in pathological autolysis, with subsequent necrosis of the cell. It has been suggested that lysosomes may be target organelles for hormones and other highly physiologically active substances.

Experimental methodology in this study includes electron microscopy, cytochemistry, autoradiography and biochemical analysis of differentially centrifugated fractions of tissue.

The aforementioned techniques, applied to hormone dependent organs, both normal and exogenously altered, during the aging process, permits investigation of morphological and chemical changes at a microcytological level. Similar methods, applied to studies of organs in which age pigments preferentially accumulate, make it possible to investigate the mode of formation of lipofuscin, its enzymic and biochemical constituents, and its possible relation to lysosomes with regard to cell aging.

HD 00086 - HD 00490  
Morris H. Ross  
Institute for Cancer Research  
Newark, Delaware

#### Effect of Changes in Enzyme Levels on Life Span

##### Aging of Cells and Tissues - Enzymatic Correlations

A series of multidisciplinary, longevity studies are being carried out on large populations of rats. They are designed (1) to determine the influence of a variety of dietary regimens upon the prevalence and severity of degenerative diseases and length of life; and (2) to determine whether modification in the rate of change in the enzyme activity patterns and of other biochemical constituents by nutritional means is intimately associated with modification in the rate of "biological aging".

Experimental, dietary regimens initiated at time of weaning are either maintained throughout life or are changed in later life. The latter provide the means of assessing the long range effects of nutrition of early life upon morbidity and mortality patterns of later life. The dietary variables are limited to protein, carbohydrate and calories, and rats are fed individually on a restricted or on an ad libitum basis. The amount of food consumed by rats fed ad libitum is determined daily.

At discrete intervals animals are chosen at random from various dietary populations and tissues taken for biochemical and cytological studies. Biochemical parameters include a number of hepatic enzymes, hepatic nitrogen, fat and water. Methods have been developed in this laboratory to determine number and dimensional characteristics of the cells of the same tissues used for the enzyme studies. These data provide an accurate method of expressing the biochemical constituents on a cellular basis. All other rats are permitted to live out their lives, and at the time of death a thorough necropsy is performed on every rat. Approximately 300,000 specimens obtained from some 7,000 rats have been thus far subjected to histopathological examination.

Disease and mortality data are analyzed by standard, actuarial procedures, and the organization of data is facilitated by computer analysis.

When the experimental diets are initiated at weaning, life expectancy patterns of the rat prove to be directly related to caloric intake. When a change in diet is initiated in the mature rat, life expectancy can also be lengthened. The duration of life, however, while greatly modified by caloric intake is dependent upon the age of the rat and the protein:calorie ratio of the diet. It appears that, since life expectancy may be modified even after growth has ceased, extension of the lifespan by dietary means might not be mediated through mechanisms involving growth retardation.

Organic disease patterns are also modifiable by dietary means and diets which effect a prolongation in lifespan also effectively reduce risk of age-associated diseases. Of particular note is the influence of the level of protein in the diet and of caloric intake on incidence and type of spontaneous tumors. While the incidence is a function of caloric intake, the type of tumor found appears to be influenced by the level of protein in the diet. In the course of these studies some 600 tumors have been found (80 separate tumor types) both benign and malignant. When the intake is high in protein and high in calories, a high incidence of tumors as well as a high proportion of malignant tumors is found. Whereas a low protein-low caloric diet results in a low incidence, a delay in time of onset and the tumors are predominantly benign.

In a similar manner a serious kidney affection in the rat, progressive glomerulonephrosis (PGN) is found to be influenced by both the level of protein in the diet and the caloric intake. PGN is most prevalent among rats that consume large amounts of protein; whereas in rats maintained on low protein-low caloric diets, the incidence is practically non-existent.

In the studies in which the experimental diets initiated post-weaning are changed, qualitatively or quantitatively, at later ages, the nutritional experiences of early life appear to have a determining influence upon physiological and pathological events of later life.

The enzyme activity levels which change with age are also modifiable by diet. The direction and degree of change are dependent upon the proportion and intake of the dietary constituents. An association among the hepatic enzyme activity patterns, life expectancy and risk to disease has been established. The manner in which the enzymes relate to life expectancy is dependent upon the basis of expressing its level of activity. Consistently, when the enzyme activities are expressed on a structural basis, either per organ or per cell, both length of life and risk of age-associated diseases correlate inversely with the level of enzyme activity.

The findings of these studies define long term metabolic influences, specifically nutritional, on the aging process at the organismic and cellular levels. They may subsequently be applicable to the nutritional aspects of aging in the human.

HD 00146  
Gerhard K. Brand  
University of Minnesota  
Minneapolis, Minnesota

#### Studies on Aging in Skin

These broad-based studies are aimed at understanding the process of aging as exemplified in the skin, both at the organismic and cellular level. The parameters observed are histological, histochemical, karyological and immunological, on young adult, old and "super-aged" mouse skin (this type is obtained by the serial transplantation of skin from animal to animal).

Research is also planned on the response of skin of various ages to infection by dermatrophic viruses, e.g., pox and Herpes simplex. Also, investigations of the influence of sex and sex steroids on skin aging will be executed in serially transferred skin grafts to determine a possible relationship between autoantibodies and skin aging. In addition, studies are being conducted on hair growth cycles and hair regenerating capacity (post-plucking) in original skin of "super-aged" skin grafts in young and old mice of both sexes, as well as in castrates. The rationale behind this section is that skin of males is thought to age faster than the skin of females.



In another series of experiments, possible correlation between tumor development and subcutaneous implantation of plastic films is sought in mice. Data in this area demonstrate a certain frequency of formation of sarcomatous transplantable tumors directly relative to subcutaneous insertion of plastic material in mice. This attracts theoretical interest as an example of "physical carcinogenesis".

HD 00261  
Morris Rockstein  
University of Miami  
Coral Gables, Florida

#### Some Physiological Aspects of Aging

This study attempts to discern specific age-related changes which take place in the enzymes involved with dephosphorylation of the adenine nucleotides, e.g., Mg-ATP-ase, as well as in the adenine nucleotide content of skeletal muscle in male Fischer and "a by c" (Sprague-Dawley) strains of rats. The Fischer rat has an average longevity of 13 months, with a maximum of 2 years, while the "a by c" strain has an average longevity of 20 months with maximums of 38 months. Concomitantly, age-related changes in the cytochrome-cytochrome oxidase system in the skeletal muscle are determined in both strains. The gastrocnemius muscle of the Sprague-Dawley strain is known to exhibit a characteristic muscular dystrophy with aging and a decrease in ATP-ase in the muscle. A pronounced decrease in total body weight also accompanies senescence, while neither ATP content nor lactic dehydrogenase exhibit any alterations. The same paradigm is effected on the aforementioned strains, which exhibit such differing lifespans, to relate aging and biochemical alterations.

Animals are sacrificed every 3 months, beginning at 6 months of age. Mg-ATP-ase as well as actomyosin ATP-ase are determined quantitatively by a standard procedure (Potter and DuBois). Inorganic pyrophosphatase and inorganic  $PO_4$  are also estimated quantitatively by several biochemical methods. Adenylate kinase, as well as cytochrome and cytochrome oxidase are determined (Potter; Keilin and Hartree; Slater). Adenine nucleotide content of the muscle homogenates is determined by differential gradient elution column chromatography.

This study may correlate cellular manifestations of senescence in an organ (skeletal muscle) known to exhibit gross generative alteration with aging. Current studies are also being carried

on the age-related distribution of creatine phosphate, the high-energy phosphagen of mammals and other vertebrates, in aging Sprague-Dawley male rat cells and muscle. Comparative studies of the distribution of both this phosphagen and the phosphagen, arginine phosphate, are also being carried out in a number of vertebrates and invertebrates.

HD 00340

George A. Talland  
Harvard University  
Boston, Massachusetts

#### Aging and the Selection of Information

Although old adult humans seem to perform less competently than young ones at most tasks, the exact dimensions of tasks along which age does or does not make a difference in performance have not been identified or analyzed adequately.

Dr. Talland is in the process of developing experimental procedures to obtain such information by testing groups of men from each age group between 20 and 70 years on a battery of perceptual and information-processing tasks. These include visual and visual-plus-auditory signal detection from continuously changing displays in which the stimulus dimensions and characteristics are varied. Learning tasks, tests involving immediate, short-term and longer-term memory and various reaction-time tests are also given.

Most of the testing proposed in the 1965 application was completed by the end of FY 1967 and new experiments were being designed to test hypotheses generated by the first round of testing.

HE 00364

Morton Lieberman  
University of Chicago  
Chicago, Illinois

#### Adaptation and Survival Under Stress in the Aged

It has long been known that the incidence of illness and death is abnormally high among the newly institutionalized elderly. This was formerly thought to be due to the fact that a noticeable downturn in health may be a major reason why the elderly person is institutionalized. However, it has also been noted that when already institutionalized elderly people are forced to move to a different ward or institution, the result is a similar, temporary elevation of illness and death rates. Thus, it is apparent that sudden environmental changes constitute a dangerous stress on the old person.

This project continues work in four lines of inquiry in this area: (1) the effects on the aged of radical socio-physical environment; (2) the psychological parameters related to the deleterious responses to disruptions in the person's life-space; (3) the mechanisms of coping developed by the aged under conditions of prolonged stress; and (4) studies of the environmental change which are particularly stressful. By the end of FY 1967, most of the data had been gathered on various experimental groups of elderly people and analysis and interpretation of data were begun.

HD 00407

Malcolm D. Jones

University of California

San Francisco, California

#### Radiographic and Pathologic Studies of Aging in the Primate Spine

This study is attempting to determine the age at which degenerative change appears in the primate spine, utilizing radiographic methods. Factors of weight bearing, torque, sex, trauma and function on the spine are under investigation. A comparison is to be drawn between the areas of maximum degeneration in the primate (gibbon) spine and human spine. Evaluation of neurologic deficit as a cause of scoliosis is under study.

A group of 11 to 15 gibbons are maintained under standard vivarium conditions. Matched controls of males and females to the animals being subjected to experimentation are maintained. All animals are subjected to periodic anesthetics, routine radiographs of the entire skeleton, and detailed radiographs of the spine including views designed to show the mobility of the spine. When adequate baseline material is available and the proper skeletal maturation pattern for satisfactory studies established, radioactive sulphur will be injected intraperitoneally (25 microcuries/kilogram body weight). Subsequent surgical removal of a lateral wedge of the vertebral bodies, intervening intervertebral discs, including nucleus pulposus, will be effected at the proper intervals (approximately 72 hours). Radioautographs and uptake counts will be made of the resected specimens. Similar studies will be conducted at approximately 10 years on the same animals to demonstrate any change in sulphur binding capacity of the nucleus pulposus.

All animals have routine radiographs of the entire skeletal system at monthly intervals during the first year of life, at three-month intervals during the second year of life, and at six-month intervals during the next three years of life. Dental counts are obtained simultaneously for correlation with the maturation pattern.

Films of the spine will be obtained at six-month intervals throughout the life of the animal, and total skeletal surveys conducted at yearly intervals to determine maturation indicators.

Use of available specimens with known birthdates provides a baseline for secondary epiphyseal ossification counts of the entire skeleton so that exact physiologic age of new specimens can be determined.

In one group of experimental animals, laminectomies are performed. In addition, unilateral two-level nerve root sections of the anterior and posterior roots are performed and in another group bilateral nerve root sections are accomplished. These operations are performed at the thoracolumbar junction and have produced gibbus formation. Marked irregularity of the development of the vertebral bodies ensues and accelerates degenerative change.

Pre-operative and post-operative opacification of the epidural plexus by way of inferior venacavography is accomplished to show the distortion of the veins around the spine as a result of surgical procedures. The change in the vascularity of the vertebral bodies is also demonstrated.

The alignment of the vertebral bodies is measured by radiographs prior to and subsequent to surgery. The presence or absence of scoliosis and abnormal vertebral development is determined in the controls. Development of osteophytes, thinning of intervertebral discs, and decreased mobility shown on the films demonstrating spine motion are criteria used in diagnosing degenerative changes of the spine.

Histologic examination and measurement of radioactivity of the biopsy specimens of the intervertebral discs and vertebral bodies provide data indicating the degree of aging and should provide a better understanding of aging processes involving the human spine.

HD 00494  
Anita Zorzoli  
Vassar College  
Poughkeepsie, New York

#### Age and Cellular Metabolism

This is a long-term study of different aspects of cellular metabolism in tissues of the mouse during aging. Under investigation is the ability of the kidney cortex to engage in gluconeogenesis, i.e. to synthesize glucose from non-carbohydrate sources.



This is being studied by measuring glucose production in slices of kidney cortex from newborn, perinatal, mature and senescent animals and by measuring the activities of six different enzymes involved in the gluconeogenesis pathway. The influence of fasting, exhaustive exercise and adrenal cortical hormones on both glucose production and enzyme activities in relation to senescence is also being investigated.

Results to date show that chronological age influences both the rate of gluconeogenesis, measured as glucose production by kidney cortex slices, and the activity of a number of gluconeogenic enzymes. At birth the specific activities of all six enzymes studied (triosephosphate isomerase, lactic dehydrogenase, fumarase, glucose-6-phosphatase, fructose-1, 6-diphosphatase and phosphoenolpyruvate carboxykinase) are well below adult levels. Phosphoenolpyruvate carboxykinase rises to over 200% of the adult value by 14 days of age and then declines. The other enzymes increase slowly, reaching adult levels by 28 days of age.

In kidney cortex tissue from senescent animals, triosephosphate isomerase, lactic dehydrogenase, fumarase and glucose-6-phosphatase activities are significantly lower than in adult tissues. Phosphoenolpyruvate carboxykinase and fructose-1, 6-diphosphatase, on the other hand, do not show age changes. The capacity for glucose production is also not diminished significantly in senescence.

Both adult and senescent animals respond to 48-hour fasting by elevation of renal glucose production and phosphoenolpyruvate carboxykinase activity. These responses are blocked by actinomycin D treatment. The data suggest that aging does not affect the ability of mouse kidney cortex cells to respond to a stimulus causing protein synthesis and that the response is quantitatively the same in senescent tissue as in adult tissue.

Adrenalectomy results in changes in activities of gluconeogenic enzymes. Specific phosphoenolpyruvate carboxykinase activity is significantly lower in adult adrenalectomized mice than in sham-operated mice. In contrast, senescent adrenalectomized mice show elevated levels of enzyme activity when compared with their sham-operated counterparts.

HD 00510  
Jack Wickstrom  
Tulane University  
New Orleans, Louisiana

#### Responses of Bone to Changes in Age and Function

This is a study of the organization and structure of compact bone and its function. The relationship between the osteones of bone

and their relationship to changes in structure as a result of alterations in function, as well as the effects of stress patterns on alterations and reorganization of the osteone system are being investigated. The hip in chickens and primates is the particular area under consideration. The stress pattern of the hip is altered by means of abnormal stress or osteotomies, after which changes in the osteone system, i.e., circulation in the neck area and head of the femur, are noted.

Rotational osteotomies are performed on the proximal femur, and the reorientation pattern at the osteotomy site after varying periods of time is observed. By using serial sections of this area, vascular changes can be followed and correlated with split-line patterns. A technique of block staining with  $\text{AgNO}_3$  of decalcified bone and gelatin mounting, is utilized in this study. Intramedullary fixation prevents derotation from recurring and interference with cortex and periosteum is kept at a minimum.

Control animals are used in which osteotomies of the femur are performed without rotation of the fragments using intramedullary fixation. It may be that altered muscular forces as well as abnormal rotation hinders the vascular supply of the head of the femur.

In another group of animals, a rubber band is attached to the ilium and proximal femur producing an adduction stress on the femur. The effects of this abnormal force on the femoral shaft and neck and head of the femur are observed.

In a third group, the effects on the architecture of the femur resulting from sectioning of the various muscle groups about the hip are noted.

Also being studied are age changes in split-line patterns in bone and their microscopic relationships in man and non-human primates, and general changes with age in bone in these subjects.

It may be of significance to correlate the findings in the chicken (with their bipedal attitude) and those in the primates, which have a skeletal system more like the human.

HD 00517  
Thomas Jones  
Angell Memorial Hospital  
Boston, Massachusetts

#### Disease in Animals Related to Aging

The aim of this project is to identify patterns and trends in disease processes in relation to aging in animals, chiefly in dogs

and cats, although other animals (birds, monkeys, horses) are included. The type and frequency of certain disease states may serve as indicators of the changes in the body which occur as a result of aging. Autopsies are performed on animals that die or are sacrificed during illness, or that have reached an old age. The anatomic findings then are correlated with age, genetic background, sex, and laboratory and clinical data which have been obtained at intervals during the lifespan of the animals. Evidence that a definite relationship exists between age and specific diseases, as well as age and diseases of particular body systems, has grown out of this study. Other correlations under study are diseases within age groups related to sex and genetic type (breed). Of particular interest among diseases having a relationship to age, breed and sex are neoplasms. The raw data from which this evidence was gained are being added to and processed for critical analysis.

A hematologic disease entity in cats (reticuloendotheliosis) and the discovery of an animal model of a human disease (canine systemic lupus erythematosus), as well as a pituitary-controlled hypothyroidism in dogs have been characterized and described by this study and is a stimulus to increase the search for additional animal models of human disease.

HD 00534  
Roy Walford  
University of California  
Los Angeles, California

#### The Role of Immune Phenomena of Aging

This study tests the concept that aging may be in considerable measure an auto-immune phenomenon. It is postulated that aging may be an immune reaction to one's own cells as they lose self markers through a process of somatic mutations. Various "model" experiments are investigated in this study. One model consists of injection of newborn animals across weak, or moderately weak, histocompatibility loci, with observation of long-term effects of this procedure upon lifespan, disease pattern and biochemical parameters. A second model consists of parabiosis across weak or moderately weak histocompatibility loci in hamsters, gerbils and mice. Changes in aging parameters are also studied in classical transplantation disease to determine whether correlations exist. Aging and chemistry in annual fishes (Cynolebias adloffi; Cynolebias bellotti) and in dwarf pygmy fish (Pandaka pygmaea) is also being investigated, for lifespan in these animals can be significantly influenced by the relatively mild environmental manipulation of reduced or elevated aquarium temperatures. Fish kept at warmer temperatures have a shorter lifespan.

All animals are followed until death, and complete records are kept to determine the incidence of various diseases in both control and experimental groups. Included in this analysis is a study of the effects of chronic immuno-suppressive therapy on lifespan, as well as experiments to test the thesis that F-1 hybrids live longer than members of the isogenic strains from which they are produced. It is hoped that some measure of somatic mutation may be obtained by studying the incidence of deviant cells in normal individuals with advancing age into the period of senility, using the Duffy and a number of other weak blood group systems. Biochemical parameters under observation in this study include: liver histidase and cathepsin; acid and alkaline phosphatase; aging pigment; cardiac succinoxidase; renal lysozyme; the ratio of soluble to insoluble collagen, as well as RNA/DNA in tissues; and the amount of fluorescent, cross-linking pigmented material in elastin and collagen of immunologically altered and aging animals.

HD 00571  
Morris Rockstein  
University of Miami  
Coral Gables, Florida

#### Aging in Musca Domestica L.

This is a study of comparative physiology and biochemistry of aging in the flight muscles of house flies. Changes at the cell level in structure, function and biochemistry in the flight muscles with aging are correlated with the characteristic decrease in flight ability and eventual failure of flight in old male flies. Cytological, histochemical and gross anatomical alterations are observed, along with changes in a number of biochemical entities, e.g., hexokinase, alpha-glycerophosphate dehydrogenase, and trehalase, in the flight muscle with advancing age. The animals are an inbred strain, maintained on a standard KLIM (dried whole bovine milk) brewers' yeast and Bacto-Agar medium. Environmental conditions (80°F and 50% RH) permit each successive generation of adults to emerge exactly 14 days subsequent to the preceding one. This permits the emergency within a few minutes, and therefore the availability, of thousands of flies of known age on one day. At known ages, the animals are exposed to CO<sub>2</sub>, separated by sex and the thoraces isolated. Sarcosomes and extrasarcosomal fractions are separated utilizing the method of Rockstein and Brandt.

Current studies have included the evaluation of the gerontomimetic characteristics of X-irradiation, administered in relatively high doses to the house fly pupal (metamorphosing) stage. These



have included the effect of high energy irradiation upon longevity of male and female flies, as well as upon the ability to fly with advancing age, as compared to normal, untreated individuals.

It has been found that within 36-48 hours prior to the onset of wing loss, there is a marked, precipitous decline in the activity of alpha-glycerophosphate dehydrogenase. Immediately concomitant with the onset of wing loss and failure of flight is the marked decline in the concentration of the intramitochondrially located Mg-activated ATP-ase, with the concomitant sudden rise in ATP content as the enzyme itself declines.

Studies of wing beat frequency have revealed that wing beat frequency in both sexes shows no appreciable change with age, but duration of flight shows a marked decline by 85% in the male at the 9th day, and in the female by 80% at the 22nd day.

Experimental surgical removal of the wings in immediately emerged adults results in no appreciable change in the age-related pattern of Mg-activated ATP-ase in the flight muscle, but does result in a sustained maximum level of alpha-glycerophosphate dehydrogenase from the 3rd day onward, in adult life.

Pupae of known age exposed to increasing x-ray doses from 2,000 to 30,000 rads showed an increase in the male to female sex ratio, from 1.04:1 at 10,000 rads to 1.32:1 at 30,000 rads.

Moreover, although the mean longevity of successfully emerging females is progressively lowered from 21 days for controls to 19 days for 10,000 rad doses to 9.1 for 30,000 rads, males, on the other hand, show a progressive increase in longevity from 13.2 days for the controls to 16 days at 10,000 rads and 17 days at 15,000 rads. However, at 20,000 rads, it is the same as the controls (13.0 days).

HD 00599  
Jerome Tobis  
Montefiore Hospital and Medical  
Center  
New York, New York

#### Study of the Physical Performance of the Elderly Patient

These are a variety of studies and programs on the rehabilitation by exercise of older and elderly patients. Much emphasis is on the patient who has cardiovascular disease. Studies in progress are:

1. Measures of cardiac output and energy cost in elderly patients of using a bedpan versus a bedside commode.
2. Measures of cardiac output during passive exercise given to legs of elderly cardiac patients.
3. Studies of the alleviation of angina pectoris by regiments which include exercise.
4. Studies of pulmonary dynamics in hemiplegics.
5. An exercise program for older businessmen who have had heart attacks, with measures of cardiac improvement. Mortality and morbidity rates will be tracked in the future.
6. Investigation of a new, computerized technique of "cardio-mapping" during rest and exercise in the elderly individual. The technique should yield much more cardiovascular information than the conventional electrocardiogram.
7. Development of instruments and methodology related to the foregoing, and determination of optimum computer programs for processing data.

HD 00636

Larry Ewing

Oklahoma State University of  
Agriculture and Applied Science  
Stillwater, Oklahoma

#### Effect of Aging and Stress on Testicular Metabolism

It is well known that in mammals both the germinal and endocrine parameters of the testis demonstrate decreased viability and function with advanced aging. This study, using rabbits, has shown that testicular aging occurs pari passu with significant alterations in testicular metabolism and secretion rate of testosterone. These data were obtained utilizing the technique of gas-liquid chromatography with electron capture detection, coupled with a unique perfused-testis system described by the principal investigator. Research is continuing in the development and adaptation of techniques for multiple steroid micro-analyses, e.g., delta-4-androstene-3, 17-dione and dehydroepiandrosterone.

Also, this study is attempting to develop or modify enzyme assays ranging from those in the hexose monophosphate shunt and

tricarboxylic acid cycle to those on the biosynthetic pathway to androgens. Specific problems being delineated in this area for each enzyme assay are optimum pH, substrate and co-factor, and also to demonstrate the dependence of initial reaction velocity upon enzyme concentration.

Specific activity of individual amino acids in testicular proteins biosynthesized in vitro is also underway.

The isolation, quantitation and scintillation counting of major end-products of glucose has already been accomplished for in vitro testis slice incubations. These techniques will be applied to the perfused testis preparation after solution of various problems encountered in modification, e.g., length of perfusion period, level of labelled glucose to be added to the system, and method of addition to the arterial blood.

Animals will be sampled at 6, 12, 18, 36 and 48 months of age and the data from these studies should elucidate effects of aging on the metabolic fate of glucose, protein, glycogen, lactic acid, DNA, RNA, and lipids in rabbit testis. Finally, the effect of aging on testosterone biosynthesis and secretion by the perfused testis will be studied, utilizing the steroid precursors acetate-1-<sup>14</sup>C and cholesterol-7- $\gamma$ -<sup>3</sup>H. The values obtained in this section will be correlated with additional information from each perfusion such as blood flow, glucose uptake, decreased amount of glucose supplied to the testis.

These experiments should aid in elaborating mechanisms involved in the detrimental structural and functional alterations occurring in the testes with aging.

HD-00642

William B. Weil, Jr.  
University of Florida  
Gainesville, Florida

#### Early Nutrition, Body Composition, and Aging

This study attempts to determine the role of early nutritional experience on the aging process. The project asks several questions: can perinatal nutrition alter the mature and senescent phases of an animal's life; can feeding patterns be altered by maternal nutrition during the intrauterine and suckling phase; and does early nutrition alter body composition in later life. The initial phases of this study were spent in establishing a rat colony and in

evaluating diets of varying protein content, colony conception rates and infant mortality rates. Three diets have subsequently been developed: 15, 30 and 60% protein, all of which give satisfactory reproductive results. Rats are now maintained on one of these three diets, after being standardized at a litter size of seven animals. There are nine sub-groups according to diet, i.e., rats given the 15% protein diet during pregnancy receive 15, 30 or 60% protein diet during lactation, etc. After weaning, animals are offered a choice of the 3 diets ad lib. These animals are observed throughout their lifetime to determine growth rate, longevity, cause of death and body composition at time of death. One group of animals is kept for breeding purposes. Rats in another group are sacrificed at intervals to determine effect of diet on body composition changes during growth. The analyses include H<sub>2</sub>O, fat, protein, ash, DNA, Na, K, Ca and Mg. Later, studies on guinea pigs are contemplated, since these animals are born in a relatively more mature state than rats. It is hoped in this way to observe the effect of early nutrition on growth, health and longevity, and on the food preferences of the post weaning animal offered a free choice of diet. Also, it is of interest whether diet pattern alters cell size and number, as well as minerals, fat and nitrogen values.

HD 00643

Clyde L. Randall

State University of New York

Buffalo, New York

#### Oophorectomy with Hysterectomy

This research deals with the retrospective study of patients with unilateral (2000), bilateral (3000), or no oophorectomy (4000) at the time of hysterectomy during the years 1928-1953. This study is conducted by analysis of the hospital records of these patients. The research group consists of 3 gynecologists, 1 epidemiologist and 1 statistician. One or both ovaries are frequently removed as a prophylactic measure when hysterectomy is indicated for benign disease. Because data are few and inconclusive, it has never been conclusively demonstrated whether or not this action can be justified.

Advantages of prophylactic oophorectomy include the obvious prevention of both benign and malignant ovarian pathology, and the possibility that ovarian removal diminishes the risk of breast and endometrial cancer.

Objections to routine or prophylactic removal of ovaries implicate the undesirable effects of castration, primarily the



degree of psychic disturbance, atherosclerosis and osteoporosis evidenced by a considerable proportion of females subjected to the effects of an abrupt and premature menopause.

After hospital records are analyzed, a follow-up study is done to determine the status of the subjects with respect to ovarian carcinoma, benign ovarian disease, all other cancer, fractures, cardiac disease, mental hospital admission and the eventual cause of death.

Another control group consists of 4000 patients with benign disease treated by gynecologic surgery other than hysterectomy.

These data should indicate the risk of preserving one or both ovaries, as well as the risk of removing them, and may be a benefit to the well-being of females who require hysterectomies for benign conditions.

HD 00648

Kurt A. Weiss

University of Oklahoma

Oklahoma City, Oklahoma

#### Tissue Changes with Aging in Highly Inbred Rats

This study involves screening and examination of the physiological and biochemical characteristics of rat tissues from animals sacrificed at different periods during their life spans. Two highly inbred strains of rats are used, the short-lived Fischer strain which has a 50% mortality rate (MR 50) at approximately 12 months, and the longer-lived AxC strain with an MR 50 of approximately 19 months.

Samples of selected tissues are taken from these animals at various stages of the life cycle and analyzed for oxygen consumption rates, electrolyte content, and hydroxyproline content. Emphasis is placed on observing the changes that occur during the life span, the time when these changes can first be observed, and the manner in which the changes progress with time.

Results show that the surviving oxygen consumption rates of liver, kidney, brain and diaphragm, expressed on a weight basis, taken from rats between the ages of 1 month and more than 2 years old, decline with age. The addition of  $1.0 \times 10^{-3}M$  pentobarbital depresses the oxygen consumption rates of all tissues studied; the percentage of the depression becomes progressively less with increasing age.

The sodium, potassium, and calcium contents of the rat femur with aging have been studied. The sodium content of the femur remains unaltered during life, while the potassium content declines rapidly during the prematuration growth phase, but remains unaltered thereafter. The calcium content increases during the growth phase and then remains relatively constant. These findings demonstrate that the greatest alterations in the bone content of electrolytes occur early in life; thereafter only relatively minor changes can be observed.

The determination of hydroxyproline content of selected tissues and organs from rats of various ages and belonging to both highly inbred strains gives an estimate of connective tissue infiltration. A gradual but significant increase of kidney, smooth muscle and brain hydroxyproline content is observed with increasing age. A lesser, but still important, increase of striated muscle and spleen hydroxyproline content is also taking place and a dramatic increase of skin hydroxyproline content is found early in life. The liver hydroxyproline content remains unchanged throughout life.

HD 00668  
Ewald W. Busse  
Duke University  
Durham, North Carolina

#### An Integrated Investigation of Aging and the Aged

This research project includes a longitudinal study that is described under long-term studies. Other components of this research project are given below with the names of the senior investigator associated with them.

S.I. Cohen  
B.M. Shmavonian  
P.L. Hein

#### Central and Autonomic Nervous System Mechanisms in Psychophysiological Responses in Young and Aged Persons

In a classical conditioning experiment young and aged subjects were studied with psychophysiological techniques. One group of studies was concerned with the influence of age on central and autonomic nervous system, neuroendocrine, and cardiovascular activity. The aged subjects have shown in comparison to young subjects less

change in adrenaline levels in the urine, less lability of beat-to-beat variation in the heart rate with respiration, peripheral vasomotor changes of smaller magnitude, and more fast low voltage electroencephalographic activity. The differences in peripheral sympathetic neurogenic and adrenaline activity and in electrocortical reactivity may reflect age determined differences in hypothalamic function. The lack of cardiovascular variability of the aged may be a function of less active efferent sympathetic discharge which could be associated with diminished variability in carotid sinus pressure and possibly a loss of the cortical inhibiting effect of carotid sinus feedback.

Differences in sensory stimulus discrimination were also noted under the conditions of the experiments which were conducted so that the young subjects showed clearer differentiation. It was also noted that within the young subject group, subjects with differences in perceptual characteristics showed differences in discrimination. Young subjects who showed poorer discrimination during a conditioning experiment and who appeared to be more stimulus bound or had a greater need for external stimuli to maintain their orientation, showed peripheral sympathetic compensatory changes similar to that noted in the aged subjects suggesting that there may be hypothalamic or other central differences within the young group which may have a genetic or maturational basis paralleling those associated with age related changes.

Animal studies have been undertaken to more clearly define the role of the carotid sinus, sympathetic and vagal fibers as well as hypothalamic areas in the regulation of cardiovascular changes particularly in response to meaningful external stimuli to which the animal must attend. The influence of respiration, cardiovascular hemodynamics and vagal blockade is being investigated.

Human studies are also being conducted in which adrenergic and cholinergic mechanisms are being explored. Young subjects (and at a later date older subjects) are being tested when alpha and beta sympathetic receptor blockade is produced. It is felt that some of the psychophysiological differences between the young and the aged may be related to the lack of autonomic feedback (which may be a function of peripheral organ changes so that they are less active). The multiple physiological effects of peripheral vascular blockade as well as the effects on cognition and perception are being studied in young subjects and will then be compared with the responses which had previously been noted in the aged subjects (without blockade). This experiment is part of a program directed at identifying the mechanisms mediating internal and external signals with psychological meaning which affect the central nervous system and in turn are associated with peripheral cardiovascular changes.

In other studies multiple physiological measurements are being obtained in experiments where primary sensory stimuli and secondary verbal symbolic signals are used as conditional stimuli. The peripheral physiological responsivity observed in relation to colors and verbal signals is different in young and aged groups. A considerable role appears to be played by the expectancy and set of the subjects in determining the response of the subjects. Attempts are being undertaken to define the relative importance of the environmental contingencies and age determined perceptions in determining situational set and subjective expectancy, and cognitive and perceptual functions in the young and aged.

The overall goal of the program is to attempt to find neurobiological and psychosocial factors associated with aging which affect complex nervous system functions.

Carl Eisdorfer

#### Verbal Learning in the Aged: Psychological and Psychophysiological Approaches

The investigative program focuses upon the study of verbal learning deficit in adults and the aged and the associated autonomic and cardiovascular events. These specific issues to be studied stem from hypotheses generated in this laboratory and elsewhere over the past few years and have been focusing on the role of internal arousal and subjective anxiety on performance during the learning situation. In addition, the effect of available time and those factors which inhibit, or facilitate responsivity in aged individuals are being studied. An attempt to modify central nervous system or autonomic nervous system status with the use of pharmacological agents is also being subjected to investigation.

George L. Maddox

#### Studies of Social Aspects of Aging and Human Development

Patterns of adaptation to retirement among selected white and blue collar workers are currently being investigated. Six years ago, in the context of a larger study of work and retirement, the work histories and attitudes of two hundred white males who were within a few years of retirement were studied. These individuals were re-interviewed after retirement and their adaptation to being out of the work force was explored. It was hypothesized that 1) satisfaction with work predicts life satisfaction in retirement



and 2) the short and long run adaptations to retirement are different for white and blue-collar workers. Specifically, the white-collar worker, who is more likely to find intrinsic rewards in his work and to experience an orderly career, brings personal and social resources to retirement which increase the probability of life satisfaction in the long run. This is the case even if, in the short run, there is a negative reaction to the loss of intrinsically valued work. In contrast, the blue-collar worker in retirement is in the short run, likely to indicate a sense of relief from extrinsically valued work; but, in the long run, the absence of work as a regulator and stabilizer of life leads to life dissatisfaction. The data support both hypotheses. This research also has provided illustrations of social rituals associated with retirement among blue-collar workers which have not been previously described in the literature.

The one-in-one thousand Census tape (1960) is also being used extensively to explore the stochastic aspects of life cycle events. Professor H.H. Winsborough is exploring the ways in which a population cohort's entry into the retirement years and movement through the terminal years of the life cycle are conditioned by the timing and ordering of prior events such as completion of schooling, marriage, work history and family size. Professor K.W. Back is studying early dropouts from the labor force, individuals between the ages of 50 and 64 who are not working and not seeking work.

Walter Obrist  
Larry W. Thompson

#### Aging and Cerebral Evoked Responses

The scalp-recorded evoked potential elicited by visual stimulation is being investigated in relation to age, background EEG activity and digital reaction time. An attempt will be made to correlate senescent changes in the "average" evoked response with deviations in the resting EEG, particularly focal disturbances, and to observe whether such changes parallel age-related increases in reaction time. Average evoked responses are determined utilizing the Computer for Average Transients which permits examination of the relationship between central nervous activity in response to peripheral stimulation, and psychological processes such as perception, learning and reaction time.

Larry W. Thompson

#### Hyperbaric Oxygenation, Behavior and EEG in the Aged

This study is designed to explore the effects of increased oxygen tensions on psychological and electroencephalographic

measures in elderly community volunteers and hospitalized patients. Subjects are exposed to three levels of inspired oxygen pressure (159 mm Hg, 760 mm Hg, and 1520 mm Hg) for approximately 30 minute intervals in a large hyperbaric research chamber. Psychological tests and clinical EEG's are completed during all exposures. The present behavior measures include a modification of the continuous performance test, reaction time tests, and the Spokes tests. Changes in psychological functioning as reflected in these measures will be compared with changes in the EEG and changes in the partial pressures of oxygen and carbon dioxide in arterial blood.

HD 00669  
Robert Kohn  
Case Western Reserve University  
Cleveland, Ohio

#### An Interdisciplinary Program of Research in Aging

This research project contains a number of individual components described below.

Howard Bensusan

#### Aging of Collagen

It is generally believed that collagen ages by formation of covalent cross-links between individual collagen molecules and between collagen molecules and other non-collagenous components of connective tissue. It is believed that aldehydic components have the capacity to link protein molecules covalently and also to link protein and mucopolysaccharide. Several such aldehyde components have been described, e.g., adipic semialdehyde (coming from a lysine residue in collagen) and enosaline (perhaps arising from lysine in collagen). Dr. Bensusan has sought to show that glucose and aldehyde can react with amino groups to form an N-glycosyl protein and that the glucose moiety can then cyclize to form 5-hydroxymethyl-2-furfural (HMF) which can link to another collagen molecule through a Mannich reaction with a histidine residue in the second collagen molecule. Model experiments have been performed, demonstrating that these reactions can occur separately in vitro and it is now proposed to look for HMF-linked collagen molecules in tropocollagen treated with glucose. Appropriately radio-labeled glucose is utilized for these purposes.

A. Burnett

Growth, Cell Differentiation, and Aging in Hydra

For many years it has been known that the cells which begin their differentiation in the hypostomal region of hydra migrate to the foot and to the tentacles. The latter are ultimately sloughed off the tip of the tentacle, either dead or soon to die. Dr. Burnett believes this is because the cells fail to secrete the collagen-like fibers of the mesogloea which constitute their substratum, and he is testing whether this is an aging effect brought about by removal of the influence of a stimulator secreted in the hypostomal region. He is testing extracts of the hypostome to see if they induce continued secretion of mesogloea at the tentacle tips. Dr. Burnett further proposes to identify the active substance, should it be found, and to perform a complete amino acid analysis and sequence study of it.

J. Edwards

Cell Death in the Insect Nervous System

Dr. Edwards is concerned with the reasons for the death of certain cells, indistinguishable from their neighbors, in the developing optic ganglion of monarch butterflies. The light microscopic evidence that these cells are moribund or dead is not entirely convincing. Dr. Edwards proposes to examine them with the electron microscope to get better evidence of their condition.

Attempts at enzyme histochemistry to identify specific changes leading to the death of these cells, particularly the localization of esterases and hydrolases, have thus far proved unsuccessful and Dr. Edwards is presently turning his attention to similar studies of programmed cell death in the cricket. He is also concerned with the electron microscopic identification of these cells, the distribution of lysosomes in them and the histo-chemical localization of esterases and cathepsins..

H. Houser

Study of the Role of Inadequate Protein Intake  
on the Development and Course of Chronic Illness

This investigator is conducting a series of studies which attempt to relate the food intake of human beings to their levels of serum cholesterol and to the fatty acid composition of adipose

tissue. Certain other serum lipids are being studied. The 56 subjects are middle-aged executives of a local brewing company.

A initial interview is held with each participant in which the recording of food intake is explained. The individual then keeps a detailed diary of all food and drink consumed during three specified days of each month for one year. The food intake data are then combined with food composition data to yield caloric intake, intake of protein, total carbohydrate, sugar, fat, vitamins and percentage of calories accounted for by animal protein, plant protein, carbohydrate, etc. No attempt has been made to estimate intake of individual fatty acids, cholesterol, etc. Biopsy of adipose tissue is performed at the beginning and at the end of the year. Serum concentrations of total lipids, cholesterol and phospholipids are determined at monthly intervals. Adipose tissue is analyzed only with respect to percentage composition of fatty acids utilizing gas-liquid chromatography.

Sidney Katz

#### Multidisciplinary Studies of Illness in Aged Persons

Dr. Sidney Katz is studying the post-hospital course of a varied group of aged persons discharged from the Benjamin Rose Hospital. His subjects include cases of hip fracture, rheumatoid arthritis and stroke, and some have already been followed for as long as ten years. The experimental design of the primary study involves a total of 300 patients subdivided into a group receiving careful post-hospital service from public health nurses and a group not receiving such a service. Within each group, half are being followed frequently and systematically; the other half are being examined only at the time of discharge and again two years later. By this design, he hopes to evaluate: (1) the effectiveness of public health nurse intervention, and (2) the consequences of the act of careful follow-up upon outcome.

Dr. Katz is collecting and coding a variety of information about many aspects of the patient's physical, social and psychological functioning by means of repeated social work home visits as well as physical examinations.

LeRoy Klein

#### Study of Skeletal Collagen Metabolism

By studies of urinary excretion of polypeptides containing hydroxyproline, Dr. Klein is determining aspects of the biosynthesis, degradation and non-destructive redistribution of collagen



in humans in growth, development and certain diseased states. Osteoporosis in particular is being studied. In conditions involving skeletal changes, data on serum alkaline phosphatase and urinary calcium are also being collected.

Dr. Klein plans to continue studies of the re-utilization of collagen. He believes that a certain amount of collagen, e.g., of skin, becomes available for reassembly into collagen granulation tissue and into collagen deposited in implanted plastic sponges. He also believes that tissue collagen, under stimulus of new areas of collagen formation, makes whole collagen molecules or large parts of these, such as alpha-chains, available for reassembly into collagen at new sites. He expects to determine under a wide variety of conditions (scurvy, inflammation, cortisone administration) what proportions of collagen are degraded, excreted as peptides, and reutilized and relate his findings to age. He will label the collagen biosynthetically with  $C^{14}$ -amino acids, stabilize the animals, and then follow the metabolic disposition of the preformed labeled collagen.

Robert Kohn

#### Aging Studies

Dr. Kohn's research on muscle atrophy in rats and mice in vivo and in vitro attempts to explore the cellular mechanisms of protein degradation. Electron microscope and isotope studies suggest that the partially degraded protein, observed in muscle subjected to lack of function, is from the myofibrillar fraction. In addition, the use of lathyrogens, e.g., beta-amino propionitrile, to inhibit cross-linking of collagen and thus retard aging is being studied.

Aaron Leash

#### Aging Colony of Rats

A colony of aging Fischer rats is maintained in isolators, pathogen-free for the purpose of supplying animals in specified age groups to investigators for experimental use. The rats are used both for collagen degradation studies and for investigations of the aging central nervous system. Similarly maintained groups of animals are used to conduct longevity studies in rats and mice with betaaminopropionitrile fumarate added to drinking water.

The aging rat colony is important for gerontological studies and has been used for investigations of age-related alterations in blood pressure and in metabolism of muscle and kidney. It is hoped that the recruitment of investigators to aging studies will be facilitated by having such animals available. Investigators not presently considering the role of age in the processes they are studying may do so if animals are available.

Discarded breeder male and female Fischer rats, Caesarean-originated, barrier-sustained, were purchased from the Charles River Breeding Laboratories, Inc., North Wilmington, Massachusetts. They were delivered in filtered cartons. The rats were introduced into pre-sterilizer 24" x 24" x 48" plastic isolators and maintained in the manner prescribed for axenic animals. In addition to rats in the specific studies mentioned above, a breeding colony is maintained and small numbers of rats of all ages will be available for studies as required.

Richard Levy

Relation of Age-Related Changes in Thyroid Hormone  
Metabolism to Gonadal Function

This is a study of thyroid function as related to the aging process. The investigators are studying the kinetics of thyroxine using several parameters in five elderly, healthy women before and after the administration of sodium gentisate, a salicylate congener.

Jerome Liebman

Effect of Restricted Neonatal Growth on Atherosclerosis  
in the White Carneau Pigeon

The influence of underfeeding in the preweaning period on development of atherosclerosis in the white Carneau pigeon is being studied by Dr. Liebman. He is assisted by Dr. Leash as co-investigator. Dr. Insull provides lipid analyses of serum, and Dr. Kohn performs pathologic examination of aortas. The project involves attempts to restrict the caloric intake of very young pigeons by placing more squabs per nest, by removing one of the parent birds, by limiting time allowed with one or more parents, etc. Body weight at six weeks is used as a measure of underfeeding. The extent and severity of aortic atherosclerosis is determined at the end of two to three years by visual inspection and by analysis for aorta cholesterol content.

Howard Schneiderman

Biological Action of Juvenile Hormone:  
Maturation and Aging in Insects

Dr. Schneiderman has a number of projects, all concerned with the endocrine control of differentiation as manifested by the specific synthesis of proteins or DNA. One of these problems is related to the obligatory intervention of DNA synthesis for de-differentiation or re-differentiation to occur. He has shown that in the Malpighian tubule cells of the adult silkworm there is no DNA synthesis. However, if the pupae are given repeated injections of juvenile hormone, DNA synthesis will begin again. This is interpreted as a de-differentiation dependent upon DNA synthesis.

In another project, Dr. Schneiderman with Dr. Narayan Patel, has been studying the blood proteins of the pupa. During prolonged perfusion, the spectrum of proteins changes and the shift suggests a reappearance of larval proteins. The conditions of the experiment are such as to make unlikely mitosis or significant DNA synthesis in time to relate to the changed protein spectrum. This is a continuing program which will be expanded to identify more clearly the new proteins as larval, to find their site of production and to extend the system to other types of protein-secreting cells.

Another main project in Dr. Schneiderman's laboratory deals with larval imaginal discs in *Drosophila*. These are transplanted into adults where half of each disc is allowed to mature to determine its capacity to differentiate and the other half is carried in an undifferentiated state. The process is continued for as many as 30 generations. During this time the cells exhibit a wide variety of differentiated cell types, many of which could not be elicited in the original disc. The project offers excellent opportunities for study of control mechanisms in differentiation.

K. Svec

Occurrence of Nuclear Reactive Factors in Sera  
of Non-Rheumatic Aged Persons

Dr. Svec, whose chief interest is systemic lupus, has developed excellent facilities for carrying out antinuclear antibody tests as well as a variety of other autoantibody procedures in that disease. She has applied these to older people and has found typically positive antinuclear reactions in 16% of people past the age of 60 compared with 3% in people 16 to 59 years of age. This observation is now being extended to other autoantibody systems.

HD 00670  
Gordon Ring  
University of Miami  
Coral Gables, Florida

A Center for the Study of Cellular Aging

This research project contains a number of individual projects described below.

L.S. Dietrich

Coenzyme Metabolism in Aged and Stressed Animals

Dr. Dietrich is studying coenzyme changes in aging animals and investigating the enzyme which is involved in the synthesis and breakdown of the coenzymes. He is utilizing animals from the two colonies of rats available from the Department of Physiology.

Bennett Sallman

Cellular Metabolic Changes in the Rat Heart with Aging

This research on cellular aging utilizing cardiac tissue from rats of two strains can be divided into two areas: (a) changes in the physical properties and biologic properties of DNA; and (b) changes in the properties of subcellular fractions from the hearts. Rat heart tissue from the AxC and Fischer strains is subjected to an exhaustive biochemical study. Among the properties being evaluated are total DNA and RNA in each fraction, nuclear and microsomal RNA, DNA base composition, templating function of extracted DNA with purified E. coli DNA polymerase, mitochondria, enzymes, etc.

George E. Schaiberger

Age-Associated Changes in Nucleic Acids  
and Proteins of E. Coli

Dr. Schaiberger is studying several aspects of the "thymine-less death" phenomenon described some years ago. In essence, the model is based upon the ultimate loss of capacity for cell division by the thymine less mutant cells after thymine deprivation. This loss of reproductive potential is time-dependent and appears to be due at least in part to incapacity of the cells to re-initiate DNA



synthesis. The change is not due to growth cessation, inhibition of DNA or protein synthesis, nor to dilution loss of DNA polymerase, since it appears before any of these things happen.

W. van Wagtendonk

#### Aging in Paramecium

Dr. van Wagtendonk has underway a program for the study of Paramecium aurelia. His research is concerned with the investigation of possible alterations in the metabolism of nucleic acids by the methods of autoradiography following exposure to C<sup>14</sup> uridine or tritiated thymidine, or both. Studies of changes with age and of the effects of radiation on aspects of aging are contemplated. Parallel studies on paramecia raised in quantity will look for more subtle effects of age on the physiocochemical properties of extracted nucleic acid and their effectiveness in enzymatic systems in vitro.

HD 00672  
Murray J. Steel  
New York University  
New York, New York

#### Multidisciplinary Study of Aging

This research project has a number of individual components that are described below.

Stanley Deutsch

#### Electrophysiologic Studies of Properties of Cell Membranes

In conjunction with the animal aging studies of Dr. Gerson T. Lesser, electrophysiological studies have been initiated to measure changes which may occur in cell membrane properties during the aging process. Specifically, changes in ionic permeability of intercostal muscle cells under conditions of osmotic stress in a young and old mouse population is to be studied in single cells with the use of microelectrodes.



Herbert Gershberg

Growth-Hormone-Parathyroid Relationships in Man  
as Measured by the Response to Acute Hypocalcemia

The interrelations of the growth and parathyroid hormones can be investigated. By infusing EDTA, a chelating agent, hypocalcemia is induced, which stimulates parathyroid hormone secretion and release of bone calcium. Treatment of adult subjects with human growth hormone is shown to accelerate the return of the blood calcium to normal after hypocalcemia.

This may be due to the effect of growth hormone on bone collagen and its calcium reserve: the collagen is made more soluble and releases its calcium reserve more readily. Compatible with this is the observation that immature rats are more resistant to hypocalcemia than more mature rats, and that older rats can be made more resistant with administration of growth hormone.

Gerson Lesser  
J. Murray Steele

Body Composition in Man; Aging and Obesity

The original procedure for independent measurement of body fat in the living human subject using the fat soluble gas cyclopropane has been improved by the substitution of Kr<sup>85</sup> during the past two years. The experimental period necessary to calculate the equilibrium has been shortened and accuracy has been increased considerably. Body fat, body water compartments and composition of the fat-free body in 47 human subjects from 16 to 90 years of age have now been observed:

1. Average body fat content at an average age of 27 was 18.9% for men and 23.8% for women. At an average age of 70 it was 26.6% for men and 37.6% for women. These values are of interest, since this is the first study (aside from a very few biochemical analyses of autopsy material) in which body fat has been directly measured. Since the mean weight of each group was within 2% of the average for the U.S. population of equivalent height, sex and age, the observed values are probably characteristic of body fat content for the current population.
2. The average water content of the fat-free body in all subjects was found to be 71.0% and to be essentially the same for both sexes throughout the adult life span.

3. Measurements of basal oxygen consumption ( $Q_{O_2}$ ) have been carried out concurrently with the independent measurements of several aspects of body composition in the same 47 human subjects aged 16-90. In aged individuals,  $Q_{O_2}$  was observed to decrease with respect to surface area, to remain constant with respect to total body water and to fat-free body mass and to increase with respect to intracellular water. Several studies provide evidence for gradual loss of cell mass with advancing age, primarily from tissues with low resting  $O_2$  uptake such as muscle.

William Perl

### Biophysical Studies

A mathematical model for the analysis of multiple indicator-dilution curves has been set up. The model is based on a previously derived partial differential equation which treats simultaneously the convective property of the vascular bed and the diffusive property of the tissue as a whole. The theory has been tested on multiple indicator-dilution experiments on dog kidney and good agreement has been found. The model allows additional physiological information to be extracted from indicator curves, such as the nature and magnitude of arteriovenous diffusion pathways.

A simplified mathematical formulation of the compartment kinetics of uptake of inert gas has been derived. The formulation has been used to derive blood flow to adipose tissue from experiments using two inert gases (krypton and cyclopropane) simultaneously. The results of their analysis support the hypothesis of direct intertissue diffusion of inert fat-soluble gases.

Simplified compartment-kinetics models have been devised, suitable for analyzing experiments on the time course of appearance and disappearance of various and variously administered substances in the blood. Applications include:

1. Continuous infusion and single injection of sulfobromophthalein (BSP), phenolsulfonphthalein (P.S.), and para-aminohippuric acid (PAH), with and without competing substances.
2. Continuous infusion and single injection of free and lipoprotein bound triglycerides into normal and diabetic rabbits.
3. Single injection of cholesterol tracer in human subjects with and without steady neomycin administration.

Joseph Post  
Joseph Hoffman

### Effect of Age Upon DNA Replication

The replication of liver cells has been studied during the life of the rat, by the use of tritiated-thymidine labeling of deoxyribonucleic acid (DNA) and autoradiography. The results show a decreasing percentage of cells engaged in DNA synthesis and mitosis from birth to maturity, at six months of age. Thereafter, from six months to three years, cell replication occurs at a very low level (less than 0.2%), indicating "wear and tear" replacement.

The diploid hepatic cell is the chief replicating unit throughout the life of the rat. Each wave of replicating cells passes through cycle after cycle of division in regular fashion. As many as four such cycles have been recorded. It is possible that during growth there is a decrease in the size of the replicating cohort and in the number of replications.

The data indicate that the DNA synthesis time remains essentially the same (6-9 hours) during the life of the rat, although at 1 day the generation time is shorter (about 14 hours) than at three weeks (21.5 hours).

These studies have been extended to ileal epithelium and spleen cells. The incomplete data show that a particular cohort of ileal cells replicates in a regular polycyclical manner. Three such cycles have been recorded before the label was "lost". These diploid cells have a generation time of 11-12 hours and DNA synthesis time of six hours. The patterns and times of replication are the same at six months as at three weeks of age.

Experiments are in progress on the DNA renewal of non-replicating tissues, for example, muscle at different times in the life of the rat. These studies were prompted by the reports of Perl which showed that non-dividing heart muscle cells are more actively replicating DNA at six months of age than at three years of age. Liver, brain, kidney and spleen are also being studied.

Maxwell Schubert

### Changes in Chemical Constitution of Cartilage with Age

Dr. Subhash Pal worked out a method to fractionate the water soluble protein-polysaccharide (PPL), which has been isolated from

bovine nasal cartilage. This method depends on the use of salts at high concentrations, PPL at very low concentration, and centrifugation at high speed. Four distinct fractions were obtained which differ in protein content, viscosity, and in sedimentation behavior. In each of these fractions protein and polysaccharide are covalently bound. The method seems applicable to human cartilage and will be used to study age changes in articular and costal cartilage.

An observation was made in this laboratory that precipitation of calcium phosphate from solution is inhibited in the presence of PPL. Evidence was accumulated that this inhibition or precipitation is due to the formation of an entity in the nature of a chemical compound consisting of microcrystals of calcium phosphate and the fraction of PPL called PPL-5. It developed that precipitation of calcium phosphate in the presence of PPL could even be used as the basis of a method to fractionate the PPL. It will be worth probing the question whether calcification in human costal cartilage, which occurs so commonly at age over 30, is related to absence of a fraction like PPL-5.

HD 00674  
Quentin Deming  
Yeshiva University  
New York, New York

#### Comprehensive Program of Research in Aging

This research project contains a number of individual projects which are described below.

I.M. Arias

#### Hepatic Excretory Function: Its Development and Senescence

This study involves metabolism of bile pigment by the liver physiologically, biochemically, cytochemically and ultrastructurally in rats, mice and humans throughout the development and aging process. Both in vivo and in vitro studies are being utilized in this research.

Peter Barland

#### Studies of Articular Tissues

This research concerns the histochemistry and microstructure of the synovial membrane and other connective tissues in degenerative states.

Paul Gallop

A Systematic Analysis of the Structure and Biosynthesis  
of Collagen

This study investigates the structure of the complex collagen molecule. A model has been suggested which may explain some of the alterations which occur during collagen maturation and aging.

Jack Geller

Pharmacologic and Endocrine Control  
of Growth of the Prostate

Preliminary data from this study suggest that suppression of gonadotrophic activity with a specific progestational compound causes regression of both normal and carcinomatous prostate glands. This research could prove to be significant regarding alleviating a common affliction in the elderly.

Phillip Henneman

Osteoporosis and the Measurement of Growth Hormone  
in the Plasma

This study concerns itself with hormonal imbalance in relation to osteoporosis. This bone disease is quite common among the elderly and may be due to an alteration in normal function of the endocrine system with aging.

Martin Liebowitz

Degenerative Renal Disease

This study is concerned with degenerative disease in the kidney and its management. It is being carried out in a group of aging men, studied both clinically and in the laboratory.

Paul Royce

Control of Organ Growth; Hypertrophy and Hyperplasia of Kidney

This study is evaluating the hypothesis that a circulating renal growth factor is responsible for the increased kidney growth which follows unilateral nephrectomy. A sensitive radiochemical



assay has been developed to investigate whether a renal growth factor can be demonstrated in peripheral blood. This study may permit a clearer evaluation of the endocrine role of the kidney, especially relating to the genesis of renal hypertension.

M.H. Williams, Jr.

#### Degenerative Pulmonary Disease of Man

This study is concerned primarily with the nature of chronic obstructive pulmonary disease and with the effects of chronic tuberculosis. The program is being effected by studying the physiology of respiration, combined with pathologic and chemical studies, particularly of the connective tissue. New histopathologic studies are being incorporated into this research.

HD 00703

Robert S. Chang

Harvard University

Boston, Massachusetts

#### Human Cells in Vitro

This is a long-term study of the phenomenon of senescence of human cells in tissue culture. Amnion cells are used since they divide infrequently. The investigator uses 14, 42 and 70-day cultures. Facts concerning aging established by this research include the observation that aging proceeds concomitantly in most experiments with an increase of cholesterol-26- $^{14}\text{C}$  and fatty acid (palmitate-1- $^{14}\text{C}$  and oleic-1- $^{14}\text{C}$ ) incorporation into cell lipids, and a decrease in their oxidation to  $\text{CO}_2$ , e.g., palmitate-1- $^{14}\text{C}$  to  $^{14}\text{CO}_2$ . Also, DNA synthesis, measured by labelled thymidine-2- $^{14}\text{C}$  and uridine -2- $^{14}\text{C}$  uptake, is decreased with aging. There is no consistent alteration in rate of synthesis of lipids from acetate-1- $^{14}\text{C}$  or glucose-6- $^{14}\text{C}$ ; in the rate of catabolism of glucose -1- $^{14}\text{C}$  and -6- $^{14}\text{C}$  to  $^{14}\text{CO}_2$ ; in the synthesis of RNA, lipids or proteins, utilizing leucine-2- $^{14}\text{C}$  and glycine-2- $^{14}\text{C}$ , or in the synthesis of RNA from uridine -2- $^{14}\text{C}$ .

Hydrocortisone at physiological concentration and extracts of Panax ginseng Korea significantly prolong the postmitotic life span of the amnion cells. Male and female sex hormones, however, are ineffective.

A relationship between aging and cell transformation has also been established. Foci of cells capable of persistent multiplication appear regularly after a long latency of 6 to 12 months. The long latent period can be shortened significantly by treating cultures with the oncogenic virus (SV40), with selected mutagens or with a chemical carcinogen.

These observations may contribute toward an understanding of senescence and the increased incidence of malignancy in old age. Also, compounds capable of prolonging postmitotic life span of cells in vitro may have similar action on cells in vivo.

HD 00769  
Freddy Homburger  
Bio-Research Institute, Inc.  
Cambridge, Massachusetts

#### Aging Studies in the Syrian Hamster

These studies are aimed at determining the life expectancy of the Syrian hamster and the aging changes that occur throughout the life span of these animals. The investigator is using about 16 inbred strains of hamsters and is observing age-influenced strain differences. Small groups of both male and female animals are autopsied periodically (21, 56 and 180 days of age) and gross and histological studies are performed on all organs. Attempts are being made to discover spontaneous disease with aging and also to develop strains which have high incidences of specific diseases. Strains that develop diseases could then serve as models for the study of human disease.

Syrian hamsters have been found to become more susceptible to infectious diseases as they grow older. New forms of constitutional disease have been observed in this study. One is an ataxia of the hind legs which begins at about one year of age and progresses to paralysis and death. This disease is confined to a single inbred strain and appears to be inherited by a sex-linked recessive gene. Preliminary histological study of the central nervous system suggests that this disorder may resemble certain degenerative diseases occurring in humans such as amyotrophic lateral sclerosis.

One inbred line, after reaching 12 months of age, develops severe obesity. It is not yet established whether this is an hereditary disorder. The incidence of cancers in the colony is extremely low; only 5 neoplasms have been observed so far. This project may eventually provide standardized inbred strains of Syrian hamsters with well-defined characteristics and perhaps with some hitherto unknown hereditary disorders.

HD 00977  
Denham Harman  
University of Nebraska  
Omaha, Nebraska

#### Effect of Antioxidants on the Life Span of LAF 1 Mice

It has been demonstrated by the principal investigator that several antioxidants, including 2-mercaptoethylamine and cysteine,

increase the average life span of C3H and AKR strains of mice when added daily to the diet throughout life. The theory behind this study is that an important aspect of the chemistry of aging is the intra- and extra-cellular damage done by free radicals in the body. These free radical molecules are highly reactive chemically due to the presence of an unpaired electron. Raising the concentration in the organism of substances capable of reacting rapidly with the free radicals theoretically might decrease their rate of attack on cellular and extra-cellular constituents and lead to a prolongation of life.

This study has been extended to male LAF<sub>1</sub> mice, a low tumor incidence strain. The antioxidants under evaluation are: 2-mercaptoethylamine; cysteine; hydroxylamine, di-tert-butyl hydroquinone; propyl gallate; butylated hydroxytoluene and 1, 2-dihydro-6-ethoxy-2, 2, 4-trimethyl-quinoline (Santoquin). These antioxidants are included in a carefully controlled purified diet fed ad lib to the LAF<sub>1</sub> mice. The effects on longevity, tumor incidence and amyloidosis are being noted. Data thus far demonstrate that 2-mercaptoethylamine, butylated hydroxy toluene (BHT) and Santoquin have significant anti-aging effects. BHT included in the diet at a concentration of 0.5% by weight increases the median survival time by 7.9 months over control animals, i.e., an increase of 53%.

HD 00988  
David Gottlieb  
University of Illinois  
Urbana, Illinois

#### Enzyme Activities in the Development of Fungi

This study is concerned with the cellular basis of aging and is a survey of various metabolic processes in certain fungi as a function of the age of cells. Growth of the fungus, Penicillium atrovenerum, measured by dry weight can be divided into the four classic phases: lag, log, stationary and death. The percentages of total nitrogen, cold TCA-soluble nitrogen, RNA and protein were found to increase to a maximum during the lag phase, subsequently decreasing as the fungus aged. The percentage of DNA was slightly higher in the spores (reproductive cells) than in the mycelium (vegetative filament). The DNA decreased in the lag phase, increased slightly to a plateau for the duration of the log phase, and then decreased to a constant percentage during the stationary and death phases. The percentage of carbohydrates in the mycelium increased continually reaching a maximum late in the log phase and subsequently decreasing as the fungus entered the

death phase. The percentages of enzyme-synthesizing compounds, e.g., amino acids, proteins, nucleotides and RNA were highest in the lag phase, whereas storage compounds such as carbohydrates increased to a maximum near the end of the log phase.

Cellular studies of the aging process continue in vegetative hyphae of Rhizoctonea solani and Sclerotium bataticola. Although respiratory activity in these organisms decreased with age, there was little decrease in activity of respiratory enzymes, e.g., glucose-6-PO<sub>4</sub>-dehydrogenase, alpha-ketoglutarate dehydrogenase, etc. Protein synthesis decreased as measured by incorporation of <sup>14</sup>C-leucine and <sup>14</sup>C-phenylalanine in whole cells. An increase was noted in the proportion of saturated to unsaturated fatty acids with age. A marked decrease in sterols occurred with age and may be indicative of cell permeability alterations with aging. Since no changes in respiratory enzyme activities were noted, it is being determined whether the reduced respiratory activity might result from a deficiency of cellular co-enzymes. Ratios and absolute levels of several co-enzymes are being determined at various ages, e.g., NAD/NADH<sub>2</sub>; NADP/NADPH; FAD; FMN; ATP/ADP; CoA; cytochromes, Mg, Mn, Zn, Fe, etc.

Since protein synthesis declines with age, cell-free protein synthesizing systems are being prepared, and will be studied to determine which step(s) in protein synthesis is initially impaired as the cell ages.

It is being determined whether the observed reduction in metabolic activity with aging, i.e., decreased respiration and protein synthesis, results from a permeability alteration of the cell to exogenous nutrients, or to accumulation and/or movement of endogenous toxic materials out of the hyphae (filaments developing from fungal germ tubes). For these observations, <sup>14</sup>C-glucose, <sup>14</sup>C-sorbose, <sup>14</sup>C-phenylalanine, <sup>14</sup>C-leucine and <sup>32</sup>P compounds are being utilized.

Cell position in a fungal colony and its effect on metabolic activity with aging are being studied. Respiration and enzyme activities are being studied utilizing several standard procedures.

HD 01066  
Gilbert Snyder  
University of Miami  
Coral Gables, Florida

#### Molecular Interactions in Skin

This investigation studies the site, number and type of inter-molecular reactions exhibited by dermal macromolecules from rats



and men of various ages. Polar and non-polar side chain groups are identified in dermal collagen, and their rates of reaction delineated into those which are free, masked and bound. The binding of hydrogen ions and various cationic and anionic dyes to tissue freed of neutral-salt-soluble tropocollagen and fat is also under investigation. Another aim of this study is to correlate elasticity and thermal degradation of connective tissue with the distribution of functional groups. The number of functional groups, elasticity and thermal degradation are expressed in terms of the clinical status of the human dermis. Particular attention is given to patients receiving anti-folic agents, thyroid and adrenocorticoid hormones, and diuretics, as well as those patients with connective tissue disorders.

HD 01179

Warren Andrew  
Indiana University  
Bloomington, Indiana

#### Age Changes in Cell Populations of Connective Tissue

Studies are being conducted on cells in connective tissue at various ages in man and laboratory animals. Work is concentrated on the connective tissue in the intestine and the dermis of the skin. Counts on the numbers of fibroblasts, endothelial cells, eosinophils, lymphocytes and plasma cells, grouped according to level of the intestine and to age of animal, have been made, utilizing grid counts for quantitation.

Results indicate a marked difference in numbers of plasma cells when very young and mature mice are compared, the numbers changing from less than five percent of the total cell population to approximately 20 percent, a proportion which is maintained into senile condition. Although eosinophils do not show a definite change with age, they do show a striking difference in numbers according to level of the intestine, averaging over 15 percent of the total number of cells in the jejunum, while in the duodenum and ileum the proportion is less than five percent. Utilizing light and electron-microscopy, total cell population in the lamina propria is not seen to vary in a significant manner with age or with level of intestine. In dermis of skin, on the other hand, cell population is far more dense in children than in adults, the proportion for infants under one year old being almost 2 to 1 as compared with young and middle-aged adults, while in senile skin the cell numbers are still lower. Fibroblasts with dense, deeply-stained nuclei tend to increase at the expense of those with clear, lightly-staining nuclei with increasing age, although this type of change is more conspicuous in Japanese than in Caucasian subjects.



Attention is now being concentrated on the relationship of the development of lymph nodules in the lamina propria to conditions in the overlying epithelium, and to the presence of micro-organisms. Individual nodules will be dissected out for study by serial sectioning or by cell dissociation. Also, bacteria will be inoculated into the lamina propria for studies on reactions. Respiratory and alimentary tract specimens of a variety of common laboratory animals will be used in this study as well as some human tissues, including prophylactically removed appendices.

Studies on rabbit appendix have shown us the constant presence of bacteria in its epithelium and lymphoid tissue after three weeks of age in normal animals.

HD 01186  
Earl B. Scott  
University of South Dakota  
Vermillion, South Dakota

#### Electron Microscope Studies of Aging

This study concerns the ultrastructure of cells and tissue of young and aged (more than 600 days) male rats, and the alterations and pathology resulting from specific dietary deficiencies. The synthetic diet consists of a mixture of 19 crystalline amino acids, vitamins, sucrose, minerals and cottonseed oil. The animals are divided into three groups, one of which receives the complete diet ad lib. Another group is fed the same diet sans phenylalanine and tyrosine, with the caloric value of the missing amino acids provided by additional sucrose. The last group is composed of rats which receive the complete diet, but are pair-fed to the deficient rats of Group 2. The normal rats provide descriptive information of the ultrastructure of young and aged rats, which serves as a base-line. All animals are sacrificed at the end of the experimental period (30 days) and a variety of tissues are prepared for study by both light- and electron-microscopy. All endocrine organs, liver, pancreas, heart, muscle, accessory sex glands, brain, kidney and intestines are utilized. A tibia from each rat is fixed in Bouins for light microscopy. For electron microscopy minute portions of tissue are fixed in buffered OsO<sub>4</sub>. Fixed tissues are then dehydrated, embedded and sectioned for light and/or electron microscopy. Staining for electron microscopy utilizes heavy metals to greater contrast; e.g., lead hydroxide, uranyl acetate, phospho-tungstic acid. Observations are made on the ultrastructure of cells during the aging process, especially kidney and thyroid and also the morphological effects of specific amino acid deficiencies on hypophyseal-thyroid, hypophyseal-gonadal and hypophyseal-bone growth relationships.

Results show that modification, in the form of basal protrusions, occurs in renal tubule cells of aged rats in areas where the peritubular basement membrane has become thickened. These changes are present in all aged rats regardless of the type, quality or quantity of their diet. It is suggested that this alteration is an attempt by the cell to compensate for an increase in resistance to transport presented by thickening of the basement membrane.

Exocrine pancreas cells of rats deprived of lysine for periods up to 90 days were examined by electron microscopy. The zymogen granules were reduced in number and exhibited fragmentation of the bounding membrane. The Golgi body was not seriously affected, and no significant changes were observed in the cell nuclei or ergastoplasm. Large clusters of lipid droplets were very conspicuous at the base of the cells. The mitochondria were swollen and deformed, exhibiting displacement of the cristae and degenerative changes resulting in myelin body formation. These alterations are considered to be the sum of effects of lysine deficiency superimposed upon the non-specific regressive effects of partial inanition.

In a group of 50 aged rats fed a complete, valine-deficient or hypocaloric (pair-fed) diet for 80 days, 60% had one or more hyperplastic nodules in one or both lobes of the thyroid. On the basis of their histology and organization, it was evident that they were derived from thyroid para-follicular cells. Formation of the tumors was related to aging, but was not influenced by the pituitary gland or by either the quality or quantity of the diets consumed.

HD 01274  
Wilma Donahue  
University of Michigan  
Ann Arbor, Michigan

#### Milieu Treatment of Older Mental Patients

Four different environments or milieus have been set up for older and elderly mental patients at Ypsilanti (Michigan) State Hospital. Two of the four wards utilize the conventional number of staff, and two have augmented staffs; two wards are oriented toward conventional hospital-custodial therapy, and two are oriented towards "community milieu" therapy. Thus the four wards may be listed as: (1) small staff-custodial, (2) small-staff community, (3) large staff-custodial and (4) large-staff community. The term "community-oriented" applies in two senses - the ward is set up as much like a real community as possible, with real responsibilities

and paid jobs (including paid work on subcontracts let by local automobile factories), and the patient is encouraged and given opportunities to have contacts with and prepare for the outside world.

For ethical reasons, the differences between conventional or "custodial" and "community oriented" wards in therapies is more a matter of degree and emphasis rather than presence versus absence of various therapeutic features.

Three objectives are being pursued: (1) the development of workable therapy and motivational techniques, tailored to the individual and designed, in the "community" version, to motivate the patient towards progressively more complex, responsible and realistic activities; (2) the measurement of the therapeutic effects of these four treatment regimens in the hospital and, for those discharged, their success in their new, less confining semi-community environments (nursing home, group residence or living in a private home); and (3) the determination of the size of staff required to produce an improved therapy system.

HD 01325  
Jack Botwinick  
Duke University  
Durham, North Carolina

#### Psychophysiological Aspects of Aging

This research deals with the general problem of loss of speed of response with advancing years and is concerned with the specific problem of the role of "set" or "expectancy" (refers to internal events inferred from 2 types of data) in this slowdown with aging.

Experiments are designed to investigate mechanisms responsible for the loss of speed with age, and the effect of set on this loss. In one group of experiments, simple auditory reaction time, utilizing a finger lift response, is compared in young (19-34 years) and old (65-84 years) male and female subjects. Electroencephalograms and electromyograms are recorded during the procedure. The same type of experiment will be subsequently performed using a visual rather than an auditory stimulus.

In a subsequent phase of the study, the following will be observed: Total as well as component parts of reaction time, electroencephalograms for young and old groups, including the subcategories of vascular disease and depression. A 1000 cps tone stimulus will be equated for effective intensity by determining

thresholds, and using various criteria of threshold, e.g., 50%, 70%, 100%, etc. Also, set will be varied by manipulating preparatory interval, and context will be varied by the use of regular and irregular series of preparatory intervals. The statistical analysis will interrelate age, sex, vascular disease and depression and these data may bear on the central nervous system locus for explaining loss of speed of response with aging.

HD 01491  
Constantinos Miras  
University of Athens  
Athens, Greece

### Aging and Alpha-Hydroxy Acid Decarboxylase of Brain

Generally, this study measures the changes with age in activity of the brain fatty acid alpha-oxidase system in rats, the properties of this system, and the occurrence of this system in other animal tissues.

In vivo studies have demonstrated that the long chain alpha-hydroxy acids of brain cerebrosides synthesized by alpha-hydroxylation of corresponding unsubstituted acids are degraded by a one-carbon process. Brain lipids are unique among lipids from other mammalian tissues since their cerebrosides contain large proportions of long-chain, normal and alpha-hydroxy fatty acids (up to C<sub>26</sub>).

This enzyme, alpha-hydroxy-acid decarboxylase, has been isolated and partially purified. Also, a necessary cofactor from the supernatant fraction has been studied utilizing high-voltage electrophoresis and ion exchange resins.

The significance of this enzymatic system may lie in its ability to degrade the long-chain acids to a chain length utilizable in the beta-oxidation system. Since it is known that in aged brains, considerably large quantities of odd-chain fatty acids are present, the connection of this enzymic system to the aging process and perhaps the breakdown of myelin is being observed.

Rat brains are studied from the period of weaning through adulthood at regular intervals, utilizing freshly prepared microsomal fractions of brain homogenates as the source of the enzyme system. Alpha-hydroxy-stearate-1-<sup>14</sup>C and alpha-keto-stearate-1-<sup>14</sup>C are substrates used, and the enzyme activity is expressed per microsomal protein nitrogen.

Experiments are in progress to characterize whether two separate enzymes or a single enzyme with multiple active sites are



responsible for the two established steps of the oxidative decarboxylation process. Since cerebrosides constitute a major lipid component of myelin, complete information on the function of this enzymic system involved with catalysis of cerebroside fatty acids in brain, with aging, may possibly correlate the demyelination process with senescence and other neurological disorders.

HD 01502

Theodore T. Tsaltas

Jefferson Medical College of Philadelphia

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### Effect of Aging and Hormones on the Cartilage Matrix

This study is concerned with the chemical composition of cartilage as it is altered by the aging process.

It has been demonstrated that the chemical composition of the cartilage matrix is significantly affected by the aging process, and that endogenously present hormones, e.g., estrogens, corticosteroids, and thyroxine retard the chemical composition changes of these tissues. Also of significance is the observation that the permeability of the cartilage matrix is decreased with aging and this decrease affects the total function of the cartilage. This may result in the degenerative changes seen in aging individuals. An experimental model has been developed to simulate aging by papain injection in rabbits. This compound alters, in vivo, the chemical composition and permeability of the cartilage matrix.

There are 3 specific aims outlined in this study. First is the determination of the composition of the cartilage matrix in male and female rabbits as a function of age. Second is the investigation of the effects of hormones (cortisone, estrogens and testosterone), and papain on the articular cartilage of mature rabbits. Third is the study of the effects of sex and aging on the permeability of the cartilage. New Zealand albino rabbits are used, grouped according to weight (corresponding to age, e.g., less than 1200 gms. - very young; 3000  $\pm$  200 gms. - adolescent, and more than 3500 gms. - mature). The cartilage plates of the ears of the rabbit are employed as the main source of cartilage. Cartilage chondromucoproteins (CMP) are extracted by either ultracentrifugation, or the standard method of Malawista and Schubert, and various chemical analyses (N, hexosamine) are carried out on the isolated CMP. Column chromatographic separation of CMP hydrolysates is also undertaken. The permeability experiments are done on articular cartilage using silver salts and labeled papain. Labeled glucose and galactose are also utilized to determine if substances essential to the nutrition of cartilage are diffusing at a different rate than that of silver salts or papain.



HD 01550  
Sidney Goldstein  
Brown University  
Providence, Rhode Island

#### Changing Consumption Patterns of the Aged

This is an analysis of data from the Bureau of Labor Statistics Survey of Consumer Expenditures of 1950 and 1960-61 which pertain to income, expenditures and savings.

The analysis aims to reveal:

1. Age differences in income, savings and consumer behavior in 1960-61.
2. Difference in income, savings and consumer patterns of the aged from 1950 to 1960.
3. The changes with age in behavior as measured by comparing behavior of the aged in 1960 with behavior of their age cohorts in 1950.
4. Study of the implications of the foregoing for the well-being of aged Americans, with attention to the probable effects of "Medicare" and other recent factors.

HD 01615  
Lissy F. Jarvik  
New York State Department of Mental  
Hygiene  
New York, New York

#### Behavioral and Chromosomal Changes of Aging

This study investigates the relations in humans among psychological, physiological and chromosomal changes with advancing adult age. The study utilizes previously studied groups, including senescent twins, a group of twins with at least one of each pair hospitalized for psychosis with chronic brain syndrome, and control groups of single-born individuals with like conditions.

Psychiatric evaluations, tests of intellectual functioning, physical and clinical laboratory medical evaluations, chromosomal sex determinations and karyotypic analyses of chromosomal material are obtained from the subjects.

The aims are: to study age-induced psychological changes, to compare the extent of chromosomal changes in senile patients with changes in like-aged non-senile persons, to evaluate involvement of

the X-chromosome as related to the sex-differential in survival time, to compare chromosomal changes in relation to test-retest changes on psychometric scores and medical measures in the periodically re-tested twins, and to determine if one-egg twin-pairs exhibit greater similarities in chromosomal changes than do two-egg twin-pairs.

HD 01691  
Stephen Griew  
University of Otago  
Dunedin, New Zealand

#### Aging and the Development of Anticipatory Responses

Although the abilities to learn and the ability to perform tasks rapidly usually are found to be lessened in the older adult human, Dr. Griew hypothesizes that as adult organisms age there is an increase in their ability to learn recurrent patterns of a statistical kind and utilize this learning in performance, so that if tasks are designed in certain ways, the older organism's performance will not be inferior to the performance of the young adult organism. This idea is being tested by a series of experiments on both humans and rats.

Studies have been completed on probability-learning and the learning of sequential patterns in humans and rats. The original hypothesis, of superior pattern-learning in the older organisms, appears to be untrue; rather the older organism tends to simplify the task for himself by attending mostly to the most frequent or regular aspects of the task. At the same time, once a statistical characteristic has been learned, older subjects tend to maintain stable performance for longer than younger ones. In other words, there appears to be a qualitative shift with age in the "strategy" used in task performance.

HD 01833  
Bryan P. Glass  
Oklahoma State University of  
Agriculture and Applied Science  
Stillwater, Oklahoma

#### Lens Weight as an Age Determiner in Freetailed Bats

This study is gaining information on the life cycle, growth and longevity of the Mexican Freetail bat (Tadarida brasiliensis mexicana). Information on the population dynamics of this species is being compiled by correlation of the lens weight (in micrograms) with

age, together with data gathered by banding and re-capturing these animals during succeeding years. The lens weights are studied in recovered specimens of 20,000 neonatally banded bats (primarily female, since they are more accessible for study) as well as animals previously banded. Lens weights from the adult spring population, when compared with those of previous years, should point out trends in survival of various age groups, and perhaps indicate changes in attrition rates from year to year on bats of various ages. Weights from samples taken later in the year may indicate survival rates of young-of-the-year, differences in behavior patterns of young vs. adults in their flight patterns, e.g., early- vs. late-emergence of certain age classes during the evening exodus, and time of migration of certain age groups. Further, age homogeneity, or lack thereof, in groups of bats flying together may be observed, as well as the proportion of young surviving to one year of age, changes in survival from year to year, and average expected lifespan. Lens weights enable the definition of age groups in bats with greater precision than previously possible using toothwear or other means. Data presently indicate an average population turn-over rate of about 8 years, with maximum longevity about 15-16 years. Overlap of weights between year classes in bats of known age is broad enough that individual bats one year old or older cannot be aged precisely by this method, but by treating large samples statistically, it is possible to determine the age structure of the population. Neonatal bats can be separated and aged precisely by weeks up to nine weeks of age, while young-of-the-year animals are separable from adults until migration in October. No gross behavioral differences between young and adults has been observed. This is an ecological study which may be applicable to age-determination of bats in general, and particularly those of medical importance that occur in sufficiently large numbers that large samples may be taken without significant alteration of the population structure.

HD 01971

Bennett Sallman

University of Miami

Coral Gables, Florida

#### Studies in the Aging of Cellular Components

This project studies effects of aging on metabolic activities of cardiac tissue in the Dutch New Zealand rabbit. The heart is utilized because it constitutes a uniformly aging population, i.e., its cells do not divide. Also, the constant contractile function presupposes a high metabolic level for the syncytially-acting constituent cells. Hearts of young (4-6 months) and old (4-7 years)

rabbits are compared utilizing several metabolic parameters, after separation of the homogenates by differential centrifugation into intact cells, nuclei, heavy and light mitochondrial fractions, and microsomal fraction (including lysosomes). The supernatant, containing nonprecipitating soluble enzymes, is also employed. Total DNA and RNA are determined, as well as nuclear and microsomal RNA.

Viscosity measurements of DNA are determined as evidence of degree of polymerization. C+G: A+T ratios of DNA (as well as C+G: A+U for RNA) are compared for young and old animals, along with studies on thermal denaturation and renaturation (regarding observations on possible cross-linking). Thymidine-2-<sup>14</sup>C is used to measure the amount of DNA synthesis, while UTP-2-<sup>14</sup>C and GTP-8-<sup>14</sup>C are utilized to measure incorporation into labeled nucleotide.

Mitochondrial fractions of young and old animals are counted, examined for swelling, and analyzed for ATP-ase activity. Energy-production mechanisms are assayed by standard techniques (O<sub>2</sub> uptake, P:O and coupling ratios) utilizing TCA cycle intermediates (succinate and alpha-ketoglutarate), lipids (beta-hydroxybutyrate, acetate and octanoate) and glutamate as substrates. Activities are also determined for malic dehydrogenase, succinic dehydrogenase and glutamic-oxalacetic transaminase.

Cathepsin and acid phosphatase levels are determined in the microsomal fraction, while glutamic-oxaloacetic transaminase and lactic acid dehydrogenase iso-enzymes are assayed in the supernatant. Comparisons with age indicate changes occur with aging in the left ventricle.

P:O and coupling ratio with tricarboxylic acid intermediates, lipids and glutamic acid as substrates show decreases with age. The most marked aging changes are in the uncoupling of oxygen uptake linked to adenosine triphosphate formation from adenosine diphosphate. Measurements of specific mitochondrial enzymes correspondingly demonstrate significantly lower activity levels.

The effects on cardiac mitochondria of administering various corticosteroid hormones (corticosterone, cortisol and cortisone) in physiological dosages to young and old rabbits are currently being studied. Reports of other investigators indicate that such steroid administration appears to decrease the age-associated deficit in left ventricular work output and that cardiac tissue is a body depository for exogenous steroid.



HD 01989  
Joseph Brinley  
St. Louis University  
St. Louis, Missouri

### Sets in the Speeded Performance of the Elderly

Older adults almost always perform tasks more slowly than young adults. This research explores the extent to which this tendency to slowness in the older subjects can be modified by variations in task memory load and in the extent to which task instructions specify the exact details of performance.

Forty-eight highly speeded tests have been administered to human subjects aged 20-36 and 59-75. The tests included variations in which the amount of memory load and the opportunity to develop expectations about the required sequence of operations are present in different combinations. Results suggest that older persons perform more efficiently when memory load is reduced and under conditions in which instructions permit subjects to anticipate the exact sequence of operations to be performed.

Further work is now under way to determine whether reduced efficiency of performance in the elderly is specifically associated with difficulty in either storing or in remembering task instructions.

HD 02033  
Neil C. Tappen  
University of Wisconsin  
Milwaukee, Wisconsin

### Structure and Function of Bone in Growth and Aging

This is an investigation of normal processes of growth, aging and structural organization of bone in man, primates (baboons, mangabeys, grivets and chimpanzees) and dogs, at gross and microscopic levels. Split-line techniques are utilized for study of structural orientation of different areas of the skeleton, and the reasons for such orientation are investigated by observations of comparable regions in different species. The split-line phenomenon involves puncture of decalcified compact bone, resulting in splits rather than holes in many regions. The orientation demonstrated by split-lines is probably explainable at a much more microscopic level than surface-parallel Haversian systems, and is probably collagenous in nature. Experimental operations and comparative studies test a variety of hypotheses as to the cause or causes of split-line orientation. Along with split-line techniques,



microscopic examination of the remodeling of compact bone is employed. Special techniques for differential block staining and serial sectioning of decalcified bone have been developed in this laboratory for tracing structures in three dimensions. In addition to allowing precise serial observations of the microscopic relationships of puncture splits to structures such as Haversian systems, these techniques aid in investigating other fundamental processes of growth and aging of bone. Specifically, resorption spaces and the formation of new bone along the developing Haversian systems can be quantitatively studied in three dimensions. Special conditions of the collagenous portion of bone matrix are studied, i.e.,  $\text{AgNO}_3$  block stain is taken up by some separate collagen bundles at a greater concentration than by others, and there are distinct topographical areas in which darkly or lightly stained bundles are congregated. A classification of cells and tissues associated with resorption spaces and vascular canals is being developed. Additional efficacious block staining techniques are being developed. Age criteria in skeletons of non-human primates are correlated with studies of arterial lesions, both endogenously and exogenously produced. The information on remodeling of compact bone is basic to an understanding of metabolic bone disease and perhaps correlation of age changes in bone with those in blood vessels.

HD 02119

William J. Felts

University of Minnesota

Minneapolis, Minnesota

#### Characteristics of Dental-Skeletal Aging

This study is developing a coherent analysis of the pattern of age-related changes that occur in the dental and general skeleton of the inbred mouse. It encompasses both macro- and micro-structure, physical characteristics of strength, mass, volume density, linear dimensions and configuration. Internal architecture of the maxilla, mandible and contained teeth, and of bone-muscle relationships will be studied from organogenesis to death at old age. The technique utilized is very high resolution radiography, and this phase of the study provides a control background for the study of alveolar healing and jaw modifications following extraction of teeth. These data are also frames of reference for information received from micro-radiographic and histological studies.

The same physical characteristics of bone are also observed in non-dental portions of the skull and in selected portions of the limb skeleton, e.g., long bones.

Cellular activity is also being observed in the form of histological and autoradiographic studies of dental and skeletal tissues and their active cellular components. In addition to an overall examination of isotope uptake and autoradiographic evidence of turnover of bone substance, particular attention is paid to the incisor, which through its characteristic of continuous growth, may reveal the dental collaries of the skeletal age changes in metabolism and production of intercellular material. This phase also acts as a control for experiments dealing with cellular and tissue responses to the trauma introduced by tooth extraction and bone fracture. Macro- and micro-radiography, autoradiography, and histology are utilized to investigate age differences in healing capacity in the alveolus after tooth extraction, and also to relate alveolar healing to parallel healing in fractures of limb bones.

A second study is the extension of these studies to the corresponding portions of the dental and general skeleton of the German Shepherd dog. In this context, the dog is a model for study of dental-skeletal aging, in which dentition is more typical of higher mammals in general. These studies may hopefully eventually be extended to man.

HD 02191

Jon J. Kabara

University of Detroit

Detroit, Michigan

#### Drugs and Brain Cholesterol During Development and Aging

This is a study of the effects of drugs on brain cholesterol in mice. Hypocholesterolemic agents and drugs known to affect brain function are utilized. The content of cholesterol in the brain is measured, as well as the conversion of labeled acetate and glucose into cholesterol. Long-range effects are studied by injecting pregnant mice and following the growth and lifespans of their progeny. Mice of both sexes aged 5, 10, 15, 20, 40, 60, 80, 160, and 320 days and on to death are used. Pregnant mice are also studied. Non-radioactive animals in some of the groups are autopsied and tissue lipid levels between controls and drug-exposed groups are compared. With the pregnant mice the dose schedule of drugs is given at various times during gestation. The progeny not receiving any drugs are studied at similar time intervals to evaluate long-range effects. Cholesterol- $^3\text{H}$  (100 micro Curies) is injected intravenously 2 days prior to the injection of sterol precursors, i.e., acetate-2- $^{14}\text{C}$ , mevalonate-2- $^{14}\text{C}$  or glucose-6- $^{14}\text{C}$ .

The mice are treated with the various drugs in single or multiple doses prior to isotope injection, at a previously determined maximum tolerated dose (MTD), 0.5 MTD, 0.1 MTD and 0.01 MTD. Among the drugs available for these studies are methylphenidate (Ritalin), 22-25 Diazacholesterol, trans-1, 4-bis (2-chlorobenzylaminomethyl) cyclohexane (AY-9944), nicotinic acid, vanadium and chlorpromazine. These compounds usually inhibit known, specific steps in cholesterol biosynthesis. Because of this, the relationship between biosynthetic events and brain development can be partially evaluated. The long-range effects of drugs on brain cholesterol levels and synthesis may be important due to a possible relationship with behavior and aging.

HD 02194  
Robert E. Anderson  
University of New Mexico  
Albuquerque, New Mexico

#### The Pathology of Accelerated Aging in Germ Free Mice

The purpose of this proposal is to investigate the contribution of bacterial and viral infections to the late effects of ionizing irradiation in mice. Specifically, it will determine whether these infections are related to the decreased longevity associated with the post-irradiation state. There is suggestive evidence that the presence of bacteria or viruses may greatly modify the direct effects of the physical agent.

The investigators began with a population of commercially obtained germ-free mice. Virgin females were chosen for this study. Half the mice were exposed at birth to bacterial flora commonly encountered by conventional mice. This variable was controlled by maintaining the animals in plastic isolators. Bacteriological and serological parameters were followed to determine whether the germ-free state was being maintained. Half the germ-free and half the germ-exposed animals were exposed to a single, whole-body, sub-lethal dose of  $\gamma$ -rays from a  $^{60}\text{Co}$  source at 6 weeks of age, and the rest of the animals in both groups served as non-irradiated controls.

The following observations between experimental groups are being evaluated: general appearance and behavior; standard hematologic data, e.g., hemoglobin, hematocrit, platelets, leukocyte, erythrocyte, and differential counts; and random gamma-globulin determinations of select animals. All animals are being allowed to live out their lifespans to determine any differences in life shortening. At death, all animals are autopsied and complete gross and microscopic tissue examination performed to establish causes of death.

Four animals from each experimental group are killed every four months. Tissues and excreta are cultured and serum specimens obtained for the determination of possible reactivity against a panel of antigens prepared from all viruses presently known to infect mice. These animals are also autopsied.

In addition, the following chemical parameters, which have been found to correlate closely with longevity, are employed: soluble/insoluble collagen in subcutaneous tissue; mucopolysaccharide ground substance/collagen in subcutaneous tissue and aorta; and age-related changes in red blood cell antigens.

If it is possible to significantly diminish the life-shortening effects of irradiation by the utilization of germ-free laboratory animals, this will be evidence that irradiation does not in fact produce a direct acceleration of physiologic aging.

HD 02207

Jean T. Snook  
Cornell University  
Ithaca, New York

#### Diet and Pancreatic Enzymes in Development and Aging

The purpose of these studies is to determine if diet can be used to modify beneficially the digestive enzyme content of the exocrine pancreas during development and aging. Initially, this study is probing the mechanisms by which various protein and amino acid diets influence enzymes of eight-week old male rats. It has been demonstrated that pancreatic enzymes secreted into the gastrointestinal tract are inactivated, digested and that the amino acids are absorbed. The presence of dietary protein in the intestine has two actions; first to increase the amounts of the enzymes secreted and second to protect them from destruction as they proceed down the small intestine. Previous work indicates that proteins most susceptible to peptic digestion provide the greatest amount of protection for pancreatic enzymes. For example, egg protein and bovine serum albumin provide protection against inactivation whereas casein does not. In addition, proteins have differential effects on the biosynthesis of enzymes in the pancreas, in that the substitution of whole-egg protein for casein in the diet causes the pancreatic content of digestive enzymes to increase. The effect of casein is retained after hydrolysis whereas the action of egg protein is modified by hydrolysis. These responses may be due in part to the superior amino acid composition of whole-egg protein and in part to its content of egg-white trypsin inhibitor (EWTI), an ovomucoid which stimulates secretion of pancreatic



enzymes and which may have other important biological effects. It is observed that pancreatic enzymes increase when methionine or EWII is added to a casein diet; pancreatic RNA also increases with addition of EWII. Since the principal goal of these studies is to determine if dietary changes can be utilized to modify beneficially the enzyme output of the pancreas during development and aging, the initial investigation concerns the relative effect on pancreatic enzyme regulation of humoral factors and of modification, by dietary and other means, of the environment in which protein synthesis occurs. This is done to observe whether the effects of a 15% protein diet can be obtained in adrenalectomized or alloxan-diabetic rats, or in rats treated with actinomycin D. Chymotrypsin, trypsin, amylase, lipase, DNA and RNA are measured. Results to date indicate adrenalectomy does not affect the relative magnitude of the pancreatic response of rats to casein and egg diets although amylase and RNA are reduced somewhat. Amylase biosynthesis is adversely affected in alloxan-diabetic rats while the pancreatic concentration of the other three enzymes is enhanced particularly when egg protein is fed. Results of experiments with actinomycin D indicate that the induction of pancreatic enzymes by dietary protein such as casein and whole-egg protein is not dependent on DNA-mediated synthesis of RNA. Studies utilizing rats ranging from the first day of life to the age of two years are in progress. Preliminary results indicate that the enzyme content of the rat pancreas falls after birth and does not reach levels measured at day one until approximately 14 or more days have lapsed. Aging rats maintained on a diet differing from the typical American diet--that is on a cereal diet containing less than 10% fat--maintain a relatively constant level of RNA and enzymes in the pancreas as they approach two years of age despite the fact they become very obese.

HD 02217  
Marie M. Jenkins  
Madison College  
Harrisonburg, Virginia

#### Relationships in Aging and Reproduction in Planarians

This study investigates possible relationships between reproductive activity and aging in the planarian, Dugesia dorotocephala, the simplest metazoan remotely similar in structure to higher animals. Planaria strains reproduce by sexual (cocoon deposition), asexual (fission) means or by an alternation of such generations. In the asexual strain, it appears that rejuvenation follows fission, and there is thus far no evidence of an aging process. In the sexual strain, by interbreeding and restricted cross-breeding experiments, two lines of descent are being established. One line



demonstrates fission prior to onset of sexuality. The other line does not demonstrate fission at all. Planaria which have become sexual do not divide unless injury occurs but show evidence of aging culminating in death. It is hoped to determine whether these characteristics are genetically controlled. The aging process in these various strains of planaria is studied with regard to longevity, cocoon production, viability of offspring and effects of fission. Changes in activity of some respiratory enzymes as a function of age are also observed. Standard biochemical procedures are utilized for the study of phosphohexose isomerase, isocitric dehydrogenase, malic dehydrogenase and aldolase. Data demonstrate that the activity of isocitric dehydrogenase is inversely proportional to the age of the planaria. Results to date also indicate that fission is an ineffective means of reproduction in the sexual strain but perhaps could be an important factor in promoting vigor in individuals which have undergone fission prior to sexual maturity.

HD 02250  
James Inglis  
Temple University  
Philadelphia, Pennsylvania

#### Age and Short-Term Memory

This study uses what has come to be known as "dichotic listening performance" (or DLP) in order to explore some of the changes that take place in human memory function with advancing age.

In DLP two independent streams of auditory information are presented to a listener, one stream to each ear through headphones off stereophonic tape. When asked to repeat what he has heard, the listener must hold the information delivered to one ear in "short-term memory storage" until he has reproduced the material read to the other ear. Thus DLP provides a way of experimenting with human short-term memory.

Dr. Inglis initiated and has continued the use of this technique for the study of age changes in human short-term memory. He has collected evidence to show that the capacity measured by DLP seems to develop from childhood to about the third decade of life and appears to decline thereafter.

In the present study Dr. Inglis is trying to answer two main questions raised by his previous work: (1) Does age primarily affect the registration or the retrieval of the material that is received to be held in short-term storage, and (2) What may be the influence of cerebral dominance of such performance.

By April 1967, two pilot studies had been completed with young and old adults. The main study is now in progress. It involves 240 subjects between the ages of 10 and 70 years, half right-handed and half left-handed. By varying the kind of material to be heard, the instructions to the listener and the kind of response required from the listener, etc., the nature of the apparent age-loss in short-term memory ability is being elucidated.

HD 02261  
Ralph F. Strebel  
New York Medical College  
New York, New York

#### Mechanisms of Tissue Calcification in Aging

The broad aim of this research is to elucidate the mechanisms underlying the medial calcific arteriosclerosis that occurs with increasing age. This problem is being studied by the experimental manipulation of two rat models of medial calcific arteriosclerosis. One model is induced chemically by dihydrotachysterol (DHT) administration for 3-4 weeks, while the other model occurs spontaneously in female breeder rats after several successive breedings.

A comparative study of the evolution of medial calcific arteriosclerosis is being made in both models. Agents which have been found to be protective against the chemical (DHT) model, e.g., ferric-dextran and methyltestosterone, are being evaluated with regard to their protective potential against spontaneously developing female breeder rat arteriosclerosis (FBRA). Non-specific stress (superimposed at various critical periods during the breeding cycle) is being investigated as a possible etiological factor in FBRA. Female rats living under germ-free conditions, pathogen-free conditions and conventional conditions ("dirty colony") are being compared with regard to the development of FBRA and DHT-induced arteriosclerosis. Lactation, plasma calcium and phosphorous levels and the role of the parathyroid and adrenal glands are also being evaluated in the etiology of FBRA. The significance of imperceptible cardiovascular damage experimentally inflicted early in life is being given particular attention as a possible causative factor in the development of spontaneous cardiovascular disease in FBRA.

HD 02304  
Charles Taylor  
Pennsylvania State University  
University Park, Pennsylvania

#### Behavioral Components of Attitudes Toward the Aging

Dr. Taylor is in the process of developing a questionnaire-scale which will predict how a person will interact socially with an older person , as well as, and in contrast to, the person's stated attitudes towards aging and older persons.

The questionnaire items utilize a technique of stating an example of social behavior with the aged and asking the respondent to state the degree to which he would be likely to interact with an older person in that way.

The subjects used include 40 graduate students with living grandparents, 40 social case-workers with case-loads which include sizeable numbers of old people, and 40 nursing-home administrators. The scale will be validated by comparing the responses of nursing-home attendants with their supervisors' ratings of how they actually behave toward their elderly charges.

HD 02377  
Marott F. Sinex  
Boston University  
Boston, Massachusetts

#### A Biophysical Study of Fluorescence in Aging Tissue

In this study, changes which occur in the elastin of aging individuals are observed. Since such elastin appears more fluorescent and more pigmented than elastin from younger subjects, a study was initiated of the unusual amino acids of elastin in an attempt to determine what structures are responsible for the pigmentation and fluorescence.

In addition to desmosine, isodesmosine and lysinonorleucine, hydrolyzates of elastin contain a number of other components not yet completely characterized. The majority of these are ninhydrin positive and absorb ultraviolet light. A strong possibility exists that some may be intermediates in the desmosine and isodesmosine biosynthetic pathway. Others may arise from the non-specific condensation of carbonyl compounds present in or about the elastin either prior to or during acid hydrolysis.

The immediate objectives are to isolate these components for characterization, and to study them histochemically in situ with an ultramicrospectrophotofluorometer designed and constructed by this group. A compound with absorption maxima at 283 Mu (in acid), and 258 Mu (in base) has proven to be a new polyfunctional amino acid. Condensation products at 283 Mu demonstrate a characteristic elastin fluorescence.

It is known that the properties of elastin in its native state change considerably during early development. The changes associated with senescence are now being studied. It is hoped to obtain some insight into the basic nature of the aging process, and particularly the cause of deterioration of the internal elastin membrane observed in aged and diseased blood vessels.

HD 02416  
Mary Adams  
Case Western Reserve University  
Cleveland, Ohio

#### Exploration of Illness Crisis in Family of the Aged

This is a study of how children or other responsible family members cope with the elderly disabled convalescent parent or relative who is dependent upon them after discharge from the hospital. Many of the elderly studied are victims of stroke or hip-fracture.

Both the elderly dependent and the responsible relative are interviewed before hospital discharge, three weeks later, and four, eight and twelve months later. The interviews aim to study:

1. Activities and strategems generated within a family in response to the crisis of a parent's unexpected immobility.
2. The family's transactions with compensatory resources within the community.
3. The value of some responses to earlier family crises in predicting the family response to the present illness crisis.



HD 02513  
Richard Hausknecht  
Mt. Sinai Hospital  
New York, New York

### Estrogen Precursors in the Post-Menopausal Female

It was discovered recently that post-menopausal women, both normal and those with endometrial carcinoma, secrete significant amounts of estrogens. However, the major estrogenic compound secreted is estrone, with little or no estradiol-17-beta being secreted. This is a reversal of the situation in normally menstruating females, in whom estradiol-17-beta is the primary estrogen and relatively little estrone is secreted. This finding suggests that urinary estrogens in post-menopausal women may be metabolic by-products of secreted steroids other than estrogens which are metabolized and conjugated. A number of C-19 compounds may serve as precursors of estradiol and estrone, e.g., dehydro-epiandrosterone and its sulfate, androstenedione, testosterone. All of these compounds can be identified in normal ovaries and adrenals. Also, adrenalectomy markedly decreases urinary estrogens in the post-menopausal female. These data suggest that the adrenals are the major source of steroids appearing in urine of post-menopausal females as estrogen conjugates and may play a relatively more important role in these females than in normally menstruating ones.

This study attempts to identify the major precursors of urinary estrogen glucuronides in post-menopausal females, and to clarify the role of the adrenals and ovaries in the production of such precursors.

$^{14}\text{C}$  or  $^3\text{H}$  labelled isotopes of estrone, androstenedione, dehydro-epiandrosterone and pregnenolone are administered intravenously into normal post-menopausal women, subjects who have had bilateral oophorectomy, women who have had adrenalectomies. Appropriate urine samples are subsequently collected. Isotope dilution is utilized to measure secretory rates and rates of interconversion of precursors, as well as of the classic estrogens. The steroid glucuronides and sulfates obtained from the urine are hydrolyzed separately and phenolic extracts obtained. The major estrogenic metabolites are identified and purified using gradient elution techniques, as well as partition column-, gas-liquid- and thin-layer-chromatography.



HD 02558  
Moe Bergman  
Hunter College  
New York, New York

#### Hearing and Aging: Description and Implications

The objectives of this study are to study the changes that occur with aging in auditory ability and the effect of such changes on the activities of daily living. The research involves the development and validation of a battery of tests which will give information on the differences in hearing between older and younger persons, the administration of the test battery to young and old subjects, and the analysis and interpretation of the data. Tests to be utilized include: threshold pure-tone air and bone-conduction audiometry, employing conventional audiometry and air conduction by Bekesy automatic audiometry. The latter includes continuous and discrete frequency tracings, both at slow table speed, and both with sustained and with pulsed tones. Also, the Short Increment Sensitivity Index (SISI) test, Fusion-Inferred Test (FIT), Rhyme test in quiet and in noise, telephone listening test, distorted speech tests, speech fusion tests and staggered word tests will be employed in this study.

There is an ample supply of subjects for study, since the research is carried out in the Metropolitan Life Insurance Company's Parkchester Housing Project, Bronx, N.Y. This project has over 40,000 residents, 12% of whom are 60 and older. This project may yield important new information concerning alteration in auditory function with aging.

HD 02566  
Joseph T. Wachsman  
University of Illinois  
Urbana, Illinois

#### Unbalanced Growth: A Molecular Basis of Death

Unbalanced growth may be induced in Bacillus megaterium by a variety of treatments including thymine deprivation on an otherwise sufficient medium, and exposure to analogues such as 5-fluorouracil or 8-azaguanine. During unbalanced growth the bacterial population dies exponentially. Conditions which permit RNA or protein synthesis to occur, enhance the bactericidal effect. Experiments in this study are designed to determine the possible involvement of each of the following, during unbalanced growth in the absence of thymine: (1) the synthesis of new classes of protein molecules, not ordinarily formed during normal exponential growth, (2) changes in the specificity of cellular regulatory mechanisms, such as degree of repression and feedback inhibition, (3) the synthesis of RNA and

DNA with altered biological properties. An understanding of the basic mechanisms involved in thymineless and analogue-induced bacterial death may provide insight into the mechanisms of cell aging.

HD 02582  
Daniel Peak  
Memninger Foundation  
Topeka, Kansas

#### Effects of Aging on Short-Term Memory

It is widely accepted that losses in short-term memory abilities tend to be characteristic of advancing adult age in humans. Dr. Peak has assembled a battery of tests, suitable for older adult humans, which measure various facets of short-term memory and also slightly longer term retention. About 10 tests have been considered at the outset. Dr. Peak is refining this battery, altering the composition and details of the tests according to preliminary results, in order to arrive at a battery of tests which will be helpful to the investigator interested in the relationship of normal aging and memory abilities. It is believed that once a refined battery is established it may prove useful to psychiatrists and other interested clinicians.

HD 02586  
Ernst Simonson  
Mt. Sinai Hospital  
Minneapolis, Minnesota

#### Physiology and Pathology of Fatigue

This project concerns the writing of a comprehensive monograph on the physiology and pathology of fatigue with documentation and critical discussion of the numerous problems involved.

The physiological changes that occur during work will be discussed, with emphasis on critical changes which may be associated with the onset of fatigue. All factors which determine performance will be discussed, i.e., constitutional variables (particularly age), environmental conditions, physical fitness, conditioning and training, and effect of various diseases. The following major sections will be included in the monograph: 1. Theory of Fatigue, 2. Age, Work and Fatigue, 3. Exercise and Fatigue in Patients. There will be a cross-coded bibliography of 6600 references.

HD 02612  
Hans Selye  
University of Montreal  
Montreal, Canada

### Studies on Tissue Development and Aging

This project is a systematic study of tissue development and aging using tissue-scaffolding and the progeria-like syndrome.

Tissue scaffolding refers to the regenerative growth of tissue in specially constructed, chemically inert tubes. Work with tissue scaffolds have shown that by varying the duration of the experiment, or the configuration and dimensions of the scaffolds, it is possible to induce the development of different tissues; e.g., bone, bone marrow, cartilage. Malignant neoplasms can also be induced with these techniques. Tubes composed of pyrex, plastic or metals do not evoke a granulomatous reaction at the implantation site. Instead, the openings are closed by a fibrin mesh, the lumen fills with an exudate, and a shred-like connective tissue cord soon crosses the central lumen, apparently growing on a preformed fibrin matrix. Modifications in the shape of the tissue scaffolds (S- or T-shaped, or spiral) is accompanied by corresponding changes in the configuration of the tissue. The present study aims at providing more information about the laws governing the induction of normal and neoplastic growth by physical means in tissues of parenchymal organs, e.g., liver, brain, lung, spleen.

Influences of humoral agents, e.g., growth hormone, glucocorticoids and anti-tumor agents on this type of metaplasia are also under observation.

A "progeria-like" syndrome (PLS) has been produced in the rat by chronic treatment with small amounts dihydrotachysterone (DHT), a calciphylactic agent. The systemic effects of calciphylaxis and its sequelae, PLS, can be modified by injecting certain challengers, e.g., ferri-dextran or Ca salts, after hypercalcemia has been induced. On the other hand, calciphylaxis and PLS are prevented when the challenger is applied prior to DHT, Vitamin D., lead or other metals. Anabolic steroids can also prevent calciphylaxis. Future research is based on the assumed central role of hypercalcemia in the production and prevention of calciphylaxis and PLS. Pertinent studies include the relation between calcium and senility, i.e., calcium and phosphorus balance studies in blood, urine and feces of rat during induction of calciphylaxis and its prevention;

the mechanism of PLS prevention by anabolic steroids; and a combined histo-chemical electron microscope study of ferri-dextran in cells of the phagocytic system. It is known that lead acetate is an effective hypercalcemic agent and that a subsequent trauma to an area of skin produces local rather than systemic calcification. Therefore, lead will be extracted from areas of injured skin and the exact site of lead deposition will be related to hypercalcemia. Further, ultrastructural studies will be effected in order to characterize the intracellular sites of lead deposition.

HD 02646  
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Yeshiva University  
New York, New York

#### Mucopolysaccharides of Connective Tissues

At present, very little is known about the biological functions of the various mucopolysaccharides. One approach to the problem is the study of the relationship of mucopolysaccharides in some inherited diseases. The urinary excretion of chondroitin sulfate B and heparitin sulfate was confirmed in over 15 cases of Hurler's syndrome, including sex-linked and autosomal recessive inheritance. It has been proposed by the principal investigator that heparitin sulfate may serve as a growth factor of connective tissue. The aim of this study is to explain the biological roles of the main groups and of their variants of the mucopolysaccharides of connective tissues, especially the chondroitin 4-, and 6-sulfates, chondroitin sulfate B, keratosulfates, and heparatin sulfates. Since most, if not all, of these sulfated mucopolysaccharides are produced by fibroblasts and related cells, the production of these complexes in tissue culture clones, and embryonal, and normal and abnormal adult tissues are studied. Special attention is being paid to the differences between the structure of keratosulfate of young and old persons. The effects of the addition of mucopolysaccharides to culture cells are studied, and their relation to collagen formation and structure, as well as the influence of pH, of O<sub>2</sub> tension, and of mineral constituents. It is assumed that some of the polyanionic mucopolysaccharides are originally cell surface constituents. The studies will also be extended to other anionic carbohydrate components of cell surfaces, especially gangliosides and sulfatides in development, aging, and inherited diseases.

HD 02718  
William Forbes  
Harvard University  
Cambridge, Massachusetts

#### The Effects of Aging on Muscular Work in Man

This is a study of the effects of aging on the capacity for muscular work in a group of men who were first observed from 1938-1941 at the Harvard Fatigue Laboratory. In that study 250 students from Harvard from 18 to 20 years old took a battery of tests of respiratory and circulatory functions in rest and during work. The present research is retesting 25 or 30 of the original subjects, repeating the same tests, and observing changes accompanying the passage of about 30 years. The results will serve as a check on data obtained in the usual "cross-sectional" study in which observations are effected at one time on persons of different ages.

Two treadmill tests are being undertaken: 1) a 6-minute walk at 3.5 mph at an 8.6% uphill grade, and 2) 3.36 mph (until exhaustion) as the grade is increased 1%/min.

Heart rate is monitored on a cardiometer during the exercise and the subsequent recovery. Vital Capacity, respiratory rate, heart rate at rest and at work and blood pressure are being observed. Post-exercise blood lactic acid is also measured as a criterion on metabolic activity in work.

HD 02721  
Vincent J. Cristofalo  
Wistar Institute of Anatomy and  
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Philadelphia, Pennsylvania

#### Carbohydrate Metabolism and Aging in Vitro

The phenomenon of aging may reflect an accumulation of externally derived insults to the organism including the effects of stresses such as disease, inadequate nutrition and radiation.



The multicellular inter-relationships of highly diversified tissues may also be involved in the aging process of the intact organisms. A question of major interest, however, is whether some inherent physiological deterioration underlies the effects of environment, i.e., whether an inherent genetic trait of the individual cells causes a physiological deterioration which may underlie any environmental or interrelative factor. This single cell senescence may be the expression of a genetically programmed delimiting of cellular viability perhaps dependent on the degree of differentiation of a particular cell type.

Cellular senescence is the subject of this proposal. The development, at the Wistar Institute, of normal human diploid cell strains in tissue culture provides a valid scientific vehicle for research in aging, since this cell strain demonstrates a finite lifespan with respect to successive generations attained during serial cultivation in vitro, usually 40 - 60 generations. Further, this lifespan is observed to terminate in a state of cellular senescence.

Two groups of in vitro cells will be utilized in this study. The first group will be those cells which retain many characteristics of normal, in situ cells, e.g., retained sex chromatin, stable normal diploid karyotype and a limited lifespan. This group includes "young" cells, i.e., those which multiply rapidly, although destined to lose this ability, and "senescent" cells, i.e., those cells which are already in the process of losing their proliferative ability.

The second group of cells has been derived from the human fetal lung cells by transformation with SV40 virus. The karyotype of this culture has been altered from the diploid and the cells have acquired the ability to propagate indefinitely.

Glucose is a central metabolite for diploid cells and since differences in energy metabolism between the young and senescent human diploid cells were demonstrated in this laboratory to be negative, the hexose monophosphate shunt pathway of glucose to pentoses and eventually to nucleic acid synthesis was chosen as

one possible locus for metabolic changes in the aging cells. The hexose monophosphate shunt provides ribose and deoxyribose for nucleic acid synthesis and also provides NADPH (or TPNH) for utilization in a number of biosynthetic reactions, e.g., fatty acid synthesis, subsequently related to membrane structure.

The experimental approach to this study involves liquid scintillation measurement of rates of incorporation of radio-active glucose into RNA and DNA in the various cell populations, and subsequent evaluation of relative contributions of the oxidative and non-oxidative pathways of pentose synthesis. Parallel measurements will be carried out on cell extracts of NADPH/NADP ratios, along with levels of activity of several enzymes involved in glucose metabolism, e.g., hexokinase, G-6-PO<sub>4</sub> dehydrogenase, transaldolase and transketolase.

The data that may be obtained with this experimental design will interrelate and correlate cell multiplication, metabolic alterations and aging in the in vitro single cell, and may subsequently provide information regarding the phenomenon of aging in the intact organism.

HD 02741  
Albert Lansing  
University of Pittsburgh  
Pittsburgh, Pennsylvania

#### Experimental Cytology of Aging Cells and Tissues

This is a broad and open-ended plan addressed to the problem of aging. The rat is the primary test animal, from two weeks of age to 1.6 years or more. Rabbits and invertebrates are also incorporated in the study.

##### A. Experimental Cytology of Aging Cells and Tissues

This study involves the enzymic activities of liver cell membranes and their changes with aging. Liver membranes are isolated and purity checked by electron microscope scanning of thin sections. The activities of the following membrane enzymes are determined: nucleoside triphosphate pyrophosphohydrolase, adenylate kinase, and an amino acid activating enzyme.

Criteria have been established distinguishing between authentic and contaminating activities of plasma membrane preparations from rat liver. In this work, a comparison was made of the specific activities and the extractabilities of eight activities in both

liver plasma membranes and liver microsomes. There is strong evidence that only four of the activities, acid and alkaline phosphatase, NAD-splitting activity, and phosphodiesterase, are components of the plasma membrane. Additional enzyme activities are also being studied, including Mg-ATP-ase, Na-K-Mg-ATP-ase, and RNA-ase. Membranes are also subjected to zonal centrifugation, and the various proteins of the membrane examined by gel electrophoresis. This is to determine any change in the component proteins with onset of maturity. The phospholipid and RNA content of the membrane is also determined.

#### B. Age and Liver Function After Partial Hepatectomy

After partial hepatectomy in rats of different ages, the uptake of  $^{14}\text{C}$ -orotate into r-RNA is measured. Also, incorporation of labeled amino acids into plasma albumin and fibrinogen is used as a further criterion. Changes observed are related to function of polysomes. To overcome pool labeling differences, ATP is isolated after labeled adenine injection, and this is related to uptake into RNA. Rate of RNA turnover is measured also by decay of isotope in the liver, kidney, and striated muscle of young and old rats.

HD 02888

Marian E. Swendseid  
University of California  
Los Angeles, California

#### Single Amino Acid Deficiencies in Aging

This project studies the effects of single amino acid deficiencies on free amino acid pools, liver RNA metabolism, and amino acid catabolizing enzymes in young and old rats. The free amino acid pools of the plasma, liver, muscle and intestinal wall are being measured during absorption of an amino acid mixture and also eight hours after forcefeeding the mixture. The RNA metabolism of the liver is being examined by measuring its total amount, its uptake of precursors, and the activity of transport RNA. The capacity of liver ribosomes to promote protein synthesis is being investigated. Typical catabolic enzymes such as tryptophan pyrolyase, tyrosine transaminase and threonine dehydratase are being assayed on the deficient diet. From this battery of tests, it is hoped to explain why, in the author's experience, essential amino acids fall into three classes, those whose deficiency causes minimal changes in plasma levels, those whose deficiency results in a reduced level of the same amino acid in the plasma, and those whose deficiency results in altered plasma levels of several amino acids.

HD 02919  
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### Age and Atrophy of Disuse

The main objective of this study is to determine the effect of age on the relation between altered endocrine function and the development of osteoporosis of disuse. One hind limb of a rat is immobilized in a plaster cast. Studies are then conducted on the effects of age, parathyroidectomy, hypophysectomy, castration, and the replacement of various hormones on bone loss and on various mechanical properties of the remaining bone. Morphologic studies of bone turnover are carried out according to the methods of Frost.

The effect of testosterone is biphasic - accentuating the degree of osteoporosis of disuse in weaning rats, but protecting against it in animals three months of age. In one group of parathyroidectomized animals the lesion protected against osteoporosis but in another of different age it had no effect. In summary, the testosterone experiment showed rather clearly, and the parathyroidectomy experiment suggested, an age-dependency of the effect of hormones on osteoporosis of disuse. These findings are being extended to other types of altered endocrine function.

NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT  
INTRAMURAL RESEARCH PROJECTS  
FISCAL YEAR 1967

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HD-AG 1  
Clyde Martin  
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Baltimore, Maryland

### Marital and Sexual Aspects of Aging

This continuing study is a systematic attempt to obtain retrospective data from older individuals concerning their experience of marriage and sexual activity, and to relate these behavioral backgrounds to a range of medical, physiologic and psychologic variables. Study objectives are as follows: (1) to collect retrospective data from longitudinal study participants in such detail as will allow all subjects to be categorized according to various aspects of their marital and sexual histories; (2) to describe variations to be found among subjects with respect to frequencies of total sexual activity in relation to age; (3) to determine the degree to which individuals maintain a relatively constant level of sexual performance over the lifespan as compared to the wide variation in sexual performance between individuals; (4) to delineate various patterns of sexual and marital adjustment and to describe interactions between these areas of adjustment; and (5) to relate the many attributes of marital and sexual experience to the numerous medical, physiologic and psychologic variables developed by other investigators at the Gerontology Research Center.

Data are obtained through highly structured interviews with the entire sample of study participants, to the extent voluntary cooperation can be secured from subjects and their memories will allow. Interviews run between 1½ to 2 hours. Questioning is conducted at the level of direct inquiry and is oriented toward collection of information that can be turned to statistical use. Priority is given to categorization of participant experience within the contexts of familial, residential, educational, military, occupational, religious, marital, and sexual behaviors, rather than to participant attitudes and personality traits.

To date, a schedule of questions has been developed to cover the major objectives of the study. These questions are also appropriate to the socioeconomic characteristics of the study population. A small number of interviews have been completed with excellent cooperation from subjects.

HD-AG 4  
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### Age Changes in Human Performance

This project is designed to study the effects of aging on (a) physiologic responses to exercise, (b) rate of recovery of

physiologic equilibrium after exercise, (c) muscular efficiency, and (d) work output and fatigue. Additionally, factors responsible for observed limitations in old people's performance are being evaluated.

Measured amounts of physical work are obtained in subjects of varying ages by means of a calibrated arm ergometer and quantitative mechanical analysis of limb movement. A treadmill is used to induce higher levels of work. Measurements of oxygen uptake, CO<sub>2</sub> elimination, pulmonary ventilation volume, heart rate, blood pressure, and cardiac output are taken before, during and after standardized amounts of exercise. Among other studies, the volume of the lungs and functional capacities of the pulmonary system are evaluated.

Through this study, a greatly simplified technique for the manufacture and use of silver-silver-chloride electrodes for measuring biopotentials in the millivolt range has been developed. This development makes possible the measurement of skin potential during an hour-long watchkeeping task. Heart rate and skin temperature measurements taken in 33 old and 33 young longitudinal participants showed a decline in vigilance (measured by percent of signals detected) for both young and old participants, but the decline was greater in older subjects. During the one hour test period, there was a significant linear increase in skin potential and decrease in heart rate; these changes were similar for both old and young participants. On the other hand, skin temperatures declined progressively in young participants but maintained a constant level in older subjects.

The uniformity of pulmonary ventilation distribution was assessed in this study in healthy men ranging in age from 20 to 103 years. Data from a study of 117 men demonstrated that ventilation was significantly less uniform in older men than in young men. Ventilation uniformity improved with increasing tidal volume in the older group only. Studies of smaller groups, in which each subject served as his own control, confirmed that old men could improve their uniformity of ventilation by increasing their tidal volumes. It was also shown that a single forced expiration prior to N<sub>2</sub> washout impaired ventilation uniformity of older men but not of the younger men. These findings indicate that older men's lungs may be more susceptible to localized alveolar collapse than those of young men.

Arm strength measurements (using a dynamometer) and manual cranking measurements (using a bicycle ergometer) were also recorded for 218 longitudinal participants. The static strength score was used to measure potential for the power generating tasks

which involved coordination of several muscle groups. The average composite strength score for arm and shoulder was 150 for participants between 20 and 69 years of age and declined to between 140 and 105 for participants in the eighth and ninth age decades, respectively. In contrast, cranking ability measured as maximum power output declined steadily for successive groups of participants older than 39 years of age. The investigators conclude that reduced coordination ability is the most likely cause of the deficit of power output relative to static strength in this study's older participants.

HD-AG 5

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#### Age Differences in Body Size and Composition

This project seeks to describe age differences in body size and composition through measurements made concurrently in individual subjects and to examine the relationship of these age differences and comparisons between subjects to physiological responses.

Subjects' height and weight are obtained by anthropometric methods. The body volume is measured by its displacement of helium in a closed chamber. Body density is calculated from these measures after correction for total skeletal mass is estimated from skinfold thickness and roentgenographic techniques. These studies are carried out on persons of differing ages and on the same people as they become older.

The physical activity for subjects participating in this study is estimated from detailed interviews with each subject and through comparison with an estimate of nutrient intake based on two one-week diet records for the 167 participants in the longitudinal aging study. The activity interviews cover activities at home, at work, at recreation (including active or passive engagement in sports), and take into account variations in activity patterns such as trips, seasonal sports, gardening, and the like. The amount of time spent in each activity is expressed as a daily average. Average calories are estimated from regression analyses of 167 participants between 20 and 99 years of age both from the activity interviews and diet diaries kept by each subject.

This project has demonstrated that there is a significant age-dependent decrease in total caloric intake which is accounted for by decrements in basal metabolism and in energy expended during physical activity.



HD-AG 6  
David Arenberg  
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### Problem Solving and Age

One general objective of this study is to determine how age is related to problem-solving performance. The experiments in this project are designed to answer such questions as are age differences a function of difficulty or type of problem, and are solution strategy-preferences related to age?

This study involves three experiments (I, IV and V) utilizing participants from the longitudinal aging study. In experiment I, the investigator uses a modified problem solving and information apparatus where each problem is displayed on a board with ten lights, nine of which have buttons to turn them on. The subject's task is to light the light which has no button (the goal light) and this must be done in a prescribed way. There is a disk with a pattern of arrows for each problem. An arrow indicates that one of three logical relations, previously explained to the subject, exists between the lights connected by arrows, but the specific relation is not known. Part of the problem is to determine the meanings of the arrows by inference or by questions of a prescribed form. The subject is told to infer as much information as he can in solving the problem. The ultimate solution of each problem is lighting the goal light by a sequence of inputs in which only a specific set of three of the nine buttons can be used. Time between inputs is controlled by the subject.

In experiment I, the subject was not required to identify the relation of each arrow on the problem disk. This is the typical procedure used in problem-solving and information problems. The subject may reach a solution with great difficulty when one or more of his implicit identifications of the relations is incorrect. This sometimes appears to be a decrement in synthesis performance when in fact, the difficulty in putting the information together appropriately should be attributed to misidentification which is an analysis error. Experiment IV is designed to avoid the confounding of analysis and synthesis performance that is possible in experiment I. What was a one-task problem in the first experiment became a two-task problem in this experiment. The first task being to identify the relations of the arrows between lights. When this is completed, the subject is given all the correct identifications and these are reviewed before he begins work on the second half of the problem--the solution. The result is that the synthesis performance (solution) is always based on accurate and complete information; and is uncontaminated by poor performance during the analysis performance (identification of relation). Several studies of concept problem solving are also included in this project.

Experiment V is a concept-problem-solving study. Conjunctive and disjunctive problems involving two foods as a solution are included in addition to simple problems with one food solutions. Examples are selected by the subject. To avoid lucky selections, the solution is not fixed and in this way logically identical selections result in the same amount of information even though the specific selections are different.

Data collection has been started for experiments IV and V. Early inspection of experiment IV data suggest that both analysis and synthesis performance appears to be poorer for older participants than for younger ones.

HD-AG 7  
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### Verbal Learning and Age

The purpose of this work is to understand age deficits in verbal learning by studying experimentally those factors which influence age differences. More immediately, the objectives are to determine age interactions which result from varying several time intervals and to evaluate the effects of inter-item interference, active responding, and various combinations of auditory and visual inputs on acquisition and retention measures.

In the first of two experiments (X and XIII), a prompting condition is compared with the typical confirmation condition. Prompting refers to a procedure in which the inspection interval precedes the anticipation interval, the reverse of the usual order. This procedure increases the probability of active responding (correctly) during acquisition trials. Timing of the interval and display of the material is accomplished by a bank of five slide projectors controlled by a cam timer. In self-paced trials, one projector is advanced by a manual switch. Each of six acquisition trials, consisting of 24 paired items is followed by a self-paced test trial. Two hours after the final test trial, a recall measure of retention is obtained using two more self-paced test trials. Subjects are low to average in vocabulary with no more than a high school education.

The other experiment (XII) is a short-term retention study with three input conditions--visual only, visual and auditory, and saying the material aloud as it appears. A film of consonant sequences and digit sequences is prepared to provide 32 test items, 6 practice items, and 4 screening items for each of the three conditions. Each digit and each consonant is shown for 0.50 seconds

(12 frames). Screening items consist of a four-digit sequence and a short interval before writing the response. These items are used to determine whether the subject (S) can retain four digits when no interfering material follows. Practice items consist of a three-consonant sequence followed by a two-second interval and another three-consonant sequence. The second group of consonants is said aloud by the S as they appear, thus serving as interfering stimuli. The S is told to write the three consonants in the first group after saying the second group of consonants. Test items are similar to practice ones, except that the first group consists of a four-digit sequence and the second is made up of a three-digit sequence. For this experiment two primary groups with no more than a high school education and a group of high education longitudinal volunteers are involved.

Preliminary analyses of data from the first experiment (X) suggests that not only does prompting at a short anticipation interval increase the probability of responding during acquisition trials (especially for the old) but the age difference is reduced under prompting conditions. In the second experiment (XII) initial analyses of data collected so far indicate that performance under the two auditory plus visual conditions is better than under the visual only condition, particularly for the older subjects. Only a small difference in performance between active and passive auditory input has emerged to date, and this difference does not appear to be greater for the old than for the young. Presently, it appears that the effectiveness for the old of responding aloud is due to the auditory input resulting and not to the activity involved in responding.

HD-AG 8  
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#### The Relations Between Age and Time Estimation Measures of Accuracy, Variability, and Context Effects

This project is aimed at determining whether age is related to simple time estimation measures (accuracy, variability, and context effects), and to what extent each of these four variables (age and the three time estimation measures) can account for performance on a more complex time judgment task involving interference from a set of interpolated stimuli.

Fourteen intervals on a log scale from 0.62 seconds to 2.14 seconds are used to make up two overlapping sets of 11 intervals each. One set is symmetrical, about one second; the other set is predominantly above one second. The subject's task is to indicate whether each interval a light is on seems longer or shorter than

one second. A total of 110 judgments (10 for each of the 11 intervals in the symmetrical set) provides a point of subjective second (PSS, a measure of accuracy) and a measure of intrasubject variability (discriminability). A PSS value is obtained also from the 110 judgments on the asymmetric set of intervals. Comparison of the two PSS values provides a measure of shift or context effects. For example, they show how much the subjective second changes with the change in the distribution of stimuli. A more complex time judgment task, which provides a measure of interference, is presented in two ways. The first requires the subject to discriminate four time periods to a certain criterion of performance, then to discriminate four intervals for 20 presentations (five of each interval) and finally to discriminate the original four intervals to the earlier criterion. Three experiments are run in all.

In experiment I, the age difference in change in errors following interpolated time judgments (interference due to retroaction) was not paralleled by an age difference in the three psychophysical measures (discriminability, accuracy of the subjective second, and context effect); but the data suggest that the correlations between each of these three measures and change in errors in the retroaction task is different for young and old.

Experiment II indicated the occurrence of an age difference in the retroaction task with the increase in errors following the interpolated judgment being greater for the old than for the young.

Experiment III substantiated almost all of the findings of experiment I. Again, the increase in errors following the interpolated judgments was greater for the old group than for the young. Also, once again the psychophysical measures showed no age differences.

HD-AG 9

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#### Age Differences in the Control of Plasma Insulin Concentration in Man and Insulin Production in Experimental Animals

This project's long range objective is to test two hypotheses dealing with the decline in glucose tolerance with increasing age-- (1) decline in tolerance is caused by relative insensitivity of the pancreatic beta cell to several stimuli including arterial glucose concentration, and (2) decline in tolerance is caused by relative insensitivity of insulin-sensitive tissues to circulating insulin.



A secondary goal of this work is the development of a new physiological technique for the study in man of the glucose-insulin closed loop system, by extrinsic servo-control of the blood glucose concentration.

Insulin is assayed for this study by a modification of the Morgan and Lazerow double antibody radio-immunoassay and plasma samples are obtained from subjects under study. The servo-control technique is used involving adjustment in rate of a continuous intravenous glucose infusion according to the difference between the actual level of arterial glucose and the desired concentration. Pancreatic islets from experimental animals are prepared by treatment of the pancreas with collagenase. Islet insulin is extracted by acid-alcohol.

To date, the complex formula for adjustment of glucose infusion rate has been simplified and improved and the critical time lag between arterial blood collection and the determination of glucose concentration with readjustment of glucose infusion rate has been reduced. Other results include the following: (1) there is a remarkable pattern of change in insulin concentration in response to the creation of a steady-state arterial glucose concentration; (2) a clear-cut dose (glucose)-response (insulin) has been defined; and (3) proof of the role of "gastro-intestinal insulin-stimulating factor" has been obtained.

HD-AG 10

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#### Age Changes in Carbohydrate Metabolism in Man

There are four major tests for diabetes in common use presently but there is little information available concerning interrelations among these tests in the same subjects and their relative sensitivity (as judged by false negatives), and specificity (as judged by false positives). This project is designed to define standards of performance on these tests of a large group of active community-dwelling men covering the entire adult span of life. Additionally, reasons for the decline in performance with increasing age are being investigated.

Subjects for this study are mainly those in the Longitudinal Study Group with the addition of some college students, laboratory and hospital personnel to increase the sample size of the group in the third and fourth age decades. Subjects are given one of the following tests per visit: oral glucose tolerance (OGTT); intravenous glucose tolerance (IVGTT); cortisone glucose tolerance (CGTT); or intravenous tolbutamide response (TRT). The cycle is restarted on the fifth visit.



Since the above tests were introduced into the testing program in 1963, over 1,200 tests have been performed. Data collected from this study have been coded and entered on punch cards for computer analyses. Manual analysis of certain aspects of the data has been undertaken including analysis of relations among the diagnostic tests. Correlations between performance of subjects on paired tests showed the highest correlation to be between performance on the IVGTT and TRT. Significant but lower correlations have been shown on the OGTT-CGTT pair and the OGTT-IVGTT pair. The lowest correlation has been obtained with the OGTT-TRT pair. The very high correlation between the IVGTT and TRT pair shows that beta cells, which are highly responsive to glucose stimulation, are also highly responsive to tolbutamide stimulation and vice versa. Further studies of this group of subjects should be informative in terms of deciding which of the two tests is more reliable in indicating an early stage of the diabetic state.

Further, in the intravenous glucose tolerance test, computation of the decay constant of glucose concentration,  $k$ , in a series of 277 subjects indicates that the  $k$  value of 1.06 (which has come to be accepted as the lower limit of normality) is unrealistic. Only 3/4ths of the young subjects, 1/2 of the middle-aged subjects, and 1/3rd of the old subjects would be classified as normal by that criterion. College students and laboratory staff, tested in order to supplement the small second and third decade age groups of longitudinal subjects, have had nearly the identical results to those found for the longitudinal study subjects of similar age.

HD-AG 12

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#### Age Changes in Cellular and Tissue Biochemistry

The general purpose of this project is to examine various tissues for metabolic changes associated with senescence. Specific investigations have included experiments to (1) study the effect of age on enzymatic activities of brain, (2) determine the effect of age on the pattern of isoenzymes in rat tissues, (3) study the effect of age on protein and nucleic acid metabolism in the rat, and (4) determine age related changes in the sulphydryl content of plasma albumin.

Acetylcholinesterase and sodium-potassium activated ATP-ase activities are measured by standard procedures in homogenates prepared from the cerebrum, cerebellum and brain stem from the right half of the brain in four-month-old and twenty-eight-month-old mice and in the cerebrum and cerebral sensorimotor cortex of 3, 6, 12, and 28-month-old female rats. In addition, the concentrations of DNA, RNA and protein are measured in these same preparations. Finally, similar sections from the left half of each brain are paraffin-embedded, sectioned and stained with a variety of stains in order to evaluate age-dependent changes in cell populations and to ascertain if the Alzheimer changes, observed in the human brain during senescence, can be detected. The quantitative patterns of the isoenzymes of lactic and malic dehydrogenases are determined in the brain, liver, kidney, heart, and muscle of rats of different ages by acrilamide gel electrophoresis.  $^{14}\text{C}$ -leucine is used to estimate protein synthesis in liver slices of 4-month-old and 28-month-old female mice and RNA synthesis is estimated in liver slices by the incorporation of either  $^{14}\text{C}$ -uridine or  $^{14}\text{C}$ -orotic acid. Plasma albumin is separated and the sulphydryl content determined.

Brain weight does not decrease with rodent senescence. There is no decrease in rat cerebrum cholinesterase with senescence. Vascular changes, gross alterations in cell distribution, demyelination, and Alzheimer's changes have not been found to occur with increasing age. A marked increase in pigmentation of the brain from adulthood to senescence has been demonstrated.

No quantitative differences have been found in the isozymes of lactic and malic dehydrogenase.

HD-AG 13

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#### Aging in the Rotifer

The purpose of this program is to determine the physiological, biochemical and histological characteristics of rotifers at various stages of their lifespans, and to determine the basic biological mechanisms responsible for factors influencing the longevity of these organisms.

This investigation uses specialized techniques for rotifer culture. Also used are standard biochemical, radioisotope assay, and histological techniques, including electron microscopy.

Culture techniques developed through this study provide the rotifers with reproducible longevity characteristics and large numbers of these animals of uniform age. Preliminary results from an electron microscope study suggest that the number of cellular organelles (ribosomes, mitochondria) decreases with age in the stomach, gastric glands and ovary of rotifers. Further studies are underway to test whether it is possible to increase the rotifers' lifespans by selecting for long-lived progeny.

HD-AG 15

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#### The Relationship of Nutrition to Biological Aging

The general purpose of this study is the investigation of nutritional influences on the rate of biological aging as estimated by changes in various biochemical parameters in tissues of the rat. Specific investigations include studies testing whether with advancing age errors occur in DNA molecules which are transmitted to RNA templates and ultimately to protein molecules, and to determine to what extent food intake limitation during lactation influences the rat lifespan.

L-ethionine-ethyl  $^{14}\text{C}$  is fed to adult rats and distribution of radioactivity is determined in proteins. RNA of liver is also determined. Two experimental procedures are used to limit rat food intake during lactation--dietary intake of the mother rat is reduced 50% during gestation and lactation, or young rats are given to mothers who have nursed another litter just prior to this time.

Study results indicate that stimulation of RNA and protein synthesis, previously noted in ethionine fed rats, may be due to two "errors"--one being the incorporation of the ethyl group into RNA by transethylation reactions. Previous data obtained on old and young rats fed a normal diet did not show changes in RNA and protein metabolism consistent with the "error theory of aging". This study has so far provided no evidence that dietary restriction in the rat during lactation results in lengthening its life in spite of a marked reduction in growth rates and final body weights of progeny.

HD-AG 21

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The Renin-Angiotensin System: Chemical-Radioisotope  
Derivative Assay of Renin and Angiotensin

This project is designed to develop a radioisotope derivative method for angiotensin, and hence for renin, which will replace the bioassay method currently in use which is exacting and limits availability of these measurements to only a few research centers. The radioisotope method would be a technique useful for both research and practical diagnosis related to various diseases which have as their common expression the development of elevated levels of blood pressure, i.e., hypertensive disease.

Previously, the investigators developed a new double isotope derivative assay for angiotensin. This work has been extended and application of the technique to measurement of angiotensin in plasma has been initiated.

This work resulted in standardization of the method to work with both angiotensin and its enzymatic conversion product, angiotensin heptapeptide, obtained by treatment with carboxypeptidase. Application to measurements in plasma was started. Modifications of the technique resulted in improved recoveries of C<sup>14</sup> indicator making it possible to routinely measure angiotensin in standard solutions in amounts as low as 1 nanogram with an error of less than 10% and a reagent blank of zero.

HD-AG 22

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Relationship of Age to Thyroid Function  
and Thyroid Hormone Metabolism

This study is an effort to learn more about the factors which control T<sub>4</sub> disposal. Previous research has shown that there is an age dependent homeostatic decrease in thyroid function which is secondary to a slowing of the mechanism(s) for the disposal (turnover) of T<sub>4</sub> (the thyroid hormone thyroxine), but the mechanism which determines this change is not understood. These studies are being conducted in subjects with experimental infections which are situations previously shown to be associated with both acceleration and slowing of T<sub>4</sub> metabolism. Specific areas under investigation



are: (a) role of plasma "free" (non-protein bound) thyroxine as a determinant of T<sub>4</sub> metabolism; (b) inter-relationships between thyroxine-binding pre-albumin (TBPA) and free T<sub>4</sub>; (c) re-evaluation of the quantitative importance of TBPA as a plasma thyroxine carrier; and (d) description of a new abnormality of thyroxine-binding globulin (TBG).

Free T<sub>4</sub> is measured by equilibrium dialysis in a dilute system utilizing magnesium precipitation of the dialysable T<sub>4</sub><sup>131</sup>. TBPA is determined using T<sub>4</sub><sup>131</sup> and the paper electrophoresis saturation technique (binding capacity) of Oppenheimer. TBG binding capacity is measured by reverse flow paper electrophoresis with T<sub>4</sub><sup>131</sup>. T<sub>4</sub> hormone turnover kinetics are measured following intravenous injections of I<sup>125</sup> labeled hormone. All subjects are prisoner volunteers from the Maryland State Penitentiary being studied by the Infectious Disease Division of the University of Maryland. Measurements are made on a daily basis before, during and after illness (for 3 to 6 weeks for each subject).

The study led the investigators to conclude that free T<sub>4</sub> is not an important regulator of T<sub>4</sub> turnover. Also, this study showed results contrary to the widely held view that TBPA is the regulator of acute changes in free T<sub>4</sub>. Re-evaluation of the quantitative importance of TBPA as a plasma thyroxine carrier indicated that given an ordinary decrease of TBPA during illness the total binding capacity of T<sub>4</sub> should not decrease by more than about 5-8%. The study turned up a new abnormality of TBG in which there is a qualitative change in the binding protein, presumably on a genetically controlled basis.

HD-AG 24

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Work Performance of Rat Heart-Lung and Isolated Heart  
Preparation: Influence of Sex and Age

The purpose of this study is to discover the mechanisms responsible for the reduced work capacity of the senescent rat heart.

Both isolated rat heart and rat heart-lung preparations are used to assess the functional capacity of the myocardium. Temperature is controlled by passing blood through a heat exchanger just before it returns to the heart. Mean blood pressure is maintained at 100 mm. Hg. in the isolated heart experiments and at 130 mm. Hg. in the heart-lung preparations. Coronary flow in



the isolated heart is measured directly from a catheter in the pulmonary artery. Arteriovenous differences are determined by obtaining simultaneous specimens of blood from the pulmonary artery and the inflow tubing from the oxygenator-reservoir. In some cases, right atrial pressure is measured by means of a saline-filled catheter inserted through the right superior vena cava into the right atrium. Electrical stimulation of the heart at a constant rate is done by means of a Grass stimulator and a bipolar silver electrode attached to the right atrial appendage. Pulse pressure curves and electrocardiograms are recorded on an Offner Dynagraph recorder.

No conclusions have been reached as yet concerning age differences with respect to glucose utilization, oxygen consumption, and lactate-pyruvate metabolism. However, blood lactate levels in both young and old rats were higher in coronary venous drainage than in arterial blood when cardiac failure was more severe, suggesting myocardial hypoxia. Coronary blood flow and the percentage of total cardiac output supplying the myocardium were usually greater in young rather than in old rats.

Heart and body weights were smaller in female rats than in male rats of the same age, but female hearts did more work and usually survived longer than did those of males, although the reason for this is still unknown. The increased incidence of cardiac arrhythmias noted in older rats seems to be one factor to explain the shorter survival and decreased work performance of older rat hearts.

HD-AG 25  
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#### Operant Responding of Rats, Hamsters, and Mice as a Function of Age

This investigation deals with determining changes in operant performance, discrimination learning and extinction as a function of age.

Animals are run in standard operant conditioning Skinner Boxes. Boxes contain bars which activate relays. Timers and feeders are standardized for animals of differing species or strains. Differences in operant level, light contingent bar pressing, operant responding for food or water rewards, extinction while nondeprived, food and/or water deprived are studied and the relations of measures obtained are compared with species, strain and age. Generally,

experiments are designed to yield the maximum information for a minimum number of animals. Animals are usually placed in 2-Bar Skinner Boxes so that light contingent bar pressing (LCBP) can be determined directly by comparisons of bar preference, rather than through the use of control groups.

Research is continuing on the analysis of light as a stimulus associated with primary reward. These stimuli are commonly termed secondary rewards. The concept of secondary reward as a confirming stimulus, providing information with respect to a correct response, has been developed to account for increments in response rates during retention tests. Studies of extinction as a function of massed versus spaced extinction trials have so far failed to produce any age differences in response retention by young and senescent male rats, although it was thought that senescent rats would fail to maintain response rates equivalent to those of young rats during a series of lengthy test trials. Tests were completed in which mature-young (5 months old), mature-old (15 months old), and senescent (25 months old male rats) were given a long series of operant trials to determine operant level, bar pressing during light and dark contingent bar press tests (L or DCBP) and relative bar preferences as a function of light intensity. Rats used were non-motivated, or motivated (tested prior to feeding). Rate differences were found to be related primarily to motivation level, with food deprived rats making more responses than non-hungry rats during all tests. In general, the two older deprived rat groups made relatively more LCBPs than DCBPs as compared with nondeprived control groups. Mature-young rat group results were in the opposite direction, confirming earlier findings that motivational differences are often difficult to obtain for young age groups.

HD-AG 26  
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#### Fluid Preferences of Rodents as a Function of Age

This work is being done to determine fluid preferences and absolute and differential gustatory thresholds as a function of age, strain and species.

Animals are run in their home cages. Calibrated drinking tubes are utilized to precisely measure fluid intake. Two bottles are within each cage to facilitate the measurement of the absolute preference threshold. One bottle contains water (standard) and

the other, for comparison, contains a given percentage of the substance for which the threshold is being obtained. Other methods involve single or multiple bottle tests, water deprivation and ingestion during or over specific time intervals.

Studies of the differential threshold (DL) for sucrose using 2 and 3 bottle tests in male rats 1, 3 and 25-months-old for a standard sucrose concentration of 9 grams, with the third bottle containing tap water in that test, showed that total fluid ingested was a function of comparison concentration and age, with senescent rats not increasing fluid intake proportionally in comparison with either young group. Fluid preferences were a function of age and number of tubes. Immature rats drank more of the higher sucrose concentration when water was available (3 tube test) than in the 2 tube test where water was not present. The 3-month old group ingested a greater proportion of the high sucrose concentration than the 1 or 27 month groups when the discrimination was very difficult. A series of tests run to assess methodological factors in sucrose preferences at high concentrations for five-month-old male rats tested at a series of sucrose concentrations at a standard concentration of 9 grams, showed that rats preferring a very high concentration of sucrose drink less total fluid than rats which prefer a comparatively low concentration, when pairs were of the range used of 9 to 27 gms/100 ml. Rats initially tested at lower concentrations continued to prefer the lower concentrations even when allowed access to higher concentrations. This project's studies of sucrose absolute thresholds and alcohol preference thresholds for food-deprived and non-deprived male albino rats (5,15 and 25-months-old) showed that normal fluid ingestion was an increasing function with increasing age and there were no differences in tube or side preferences as a function of age or deprivation. Fluid ingestion of deprived rats increased when above threshold concentrations of sucrose were presented, but there were no differences in proportion of sucrose ingested between deprived and non-deprived groups at any age level. Sucrose discrimination was shown to be a decreasing function with increasing age. Alcohol preference was lower for the 15-month-old non-deprived rats than for the other non-deprived groups, but the effect of food deprivation resulted in a proportionately greater difference in alcohol preference for the 15-month-olds than for the 5 or 25-month old groups (deprived vs. non-deprived).

HD-AG 27  
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An Experimental Analysis of Rodent Exploratory Behavior  
as a Function of Age

This project seeks to develop reliable and valid tests for measuring exploration, to determine the amount of exploratory behavior under specified conditions as a function of age and heredity, and to specify variables which control such behavior.

Time sample methodology is utilized for all experiments. For the present work several different test areas and time intervals are used. An environment is developed which allows measurement of normal behavior such as sniffing, grooming, or lying, as well as preference for light or comparative darkness.

Consecutive tests of male albino rats showed that open field locomotor activity was higher for deprived than for non-deprived rats at all ages. Emotionality scores connected with locomotor activity, showed that non-deprived rats were more emotional than deprived rats with emotionality increasing directly as a function of increasing age. Emotionality of senescent rats stayed at a relatively high level, decreased for mature-old rats, and remained at relatively low levels for mature-young rats. Similar results were obtained in tests of exploration and emotionality. Wheel activity was higher for deprived than non-deprived rats and was a decreasing function with increasing age. Tests run with young and senescent female inbred mice showed similar scores to those for rats in exploration and activity. The data obtained indicate that inbred mice might be used in psychogenetic experiments.

HD-AG 28  
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Structure of Nucleic Acids

This project seeks to study the reactions of metal ions, proteins and synthetic polypeptides with nucleic acids; to find specific reactions of the individual nucleotides; to understand the biological significance of metal ions in nucleic acid function; and to develop methods for determining nucleotide sequence of nucleic acids.



Conditions are determined for the binding of metal ions to (1) the phosphates on polynucleotide chains and (2) the bases attached to the ribose phosphate backbone. Metal binding to phosphate is used to degrade the polymers, and the degradation reactions are studied for possible specificities in their attack on different inter-nucleotide bonds. Metal binding to the bases competes with the hydrogen bonding of these bases and thus affects the helix-coil transitions of the polymers. Protein binding to the phosphates simulates a condition of high electrolyte concentration. Preferential binding of metal ions to some of the bases can be used in sequential analysis either by rendering the affected bases more susceptible to hydrolytic attack or by specifically inhibiting the attack of nuclease enzymes upon the metal bound nucleotides.

Addition of ATP to DNA in equimolar ratio (of nucleotides) resulted in hypochromicity and a substantial decrease in the melting temperature of DNA; meaning that DNA serves as a template on which nucleoside triphosphate molecules are stacked, and the tetranegative charge of the ATP anion destabilizes the double helix of DNA, causing its unwinding.

Previously it was shown that in the presence of Cu (II) ions, the double-stranded form of DNA is destroyed at low ionic strength and restored at high ionic strength. It was discovered that zinc ions can be used to reversibly unwind and rewind double-helical DNA by heating and cooling, respectively, and that this process can be repeated indefinitely, resulting always during the rewinding step, in the complete renaturation of the DNA.

Earlier work on the effect of metals on the  $T_m$  of DNA showed that some metals decrease  $T_m$  and others increase it, depending on whether the metals bind to the bases or the phosphate, respectively. It has been further shown from the dependence of the  $T_m$  of DNA upon the metal concentrations that probably most metals bind somewhat to both bases and phosphate, with vastly differing relative strength.

A systematic study of the interaction of metal ions with nucleohistone complex, which may play a role in cell differentiation and aging phenomena in multicellular organisms, has been started.

Methods for the cleavage of phosphodiester bonds in RNA with zinc ions and the determination of the cleavage results have been reported before. Also, it has been indicated in prior studies that cleavage occurs least readily at bonds adjacent to guanine bases. It has not been found that the lack of cleavage at guanine is compensated by higher cleavage at uracil. This cleavage reaction



can be extremely important if it can be made quantitative. If this can be done, the zinc degradation reaction can be used like an enzymatic reaction in nucleotide sequence studies.

Indications that a histone fraction from bovine thymus is unusually strongly bound to nucleic acid, and that the quantity of this particular histone fraction increases with age, has been shown earlier. Similar fractionization of histone from nucleoprotein isolated from rat liver has now begun.

HD-AG 30

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#### The Function of Metal Ions in Enzymatic Reactions

This investigation is an attempt to understand the mechanisms by which metal ions participate in enzymatic reactions, and to determine why different metal ions are required for different enzymatic processes.

Selected enzymatic substrates are treated with metal ions under varying conditions, and the nature of the metal-substrate interactions is detected by physical measurements such as spectrophotometry. Interaction of the metals with enzyme protein is studied by macromolecular techniques such as ultracentrifugation.

The investigators report that there appears to be little question that the DNAase reaction with Cu (II) produces the same kind of specificity in cleaving DNA as the zinc reaction produces with RNA: i.e., preferential cleavage occurs at the thymine sites, while the guanine sites are much less readily attacked.

A review of macromolecular coordination compounds in biological systems as been completed.

HD-AG 31

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#### Studies on Mitochondrial Energy Metabolism

These studies are to dissociate and purify some factors associated with mitochondrial oxidative and energy coupling reactions, and reconstitution of the system involved in the energy-transduction process with discrete components of known function.

Two mitochondrial reductases are purified by chromatography and ammonium sulfate fractionization. Sedimentation properties are re-evaluated by ultracentrifugation. Coupling Factor A is purified by DEAE - cellulose column chromatography, and sucrose density gradient centrifugation. Coupling Factor B is purified by similar methods with the addition of ammonium sulfate fractionization and CM - cellulose column chromatography. Groups of rats are surgically thyroidectomized, and after a 6-8 week period, liver mitochondria are prepared from these animals.

DPNH-Ubiquinone reductases were further purified and characterized. Purification and characterization of two coupling factors (A and B) was carried out and, in specially prepared ammonia-treated submitochondrial particles, Factor A was found to have almost no effect in restoring P/O or succinate reversal activity, while Factor B was maximally effective in restoration. Liver mitochondria prepared from surgically thyroidectomized rats was assayed to determine oxidative activities, P/O, respiration control, thyroxin sensitivity, and sensitivity to various respiratory inhibitors, including oligomycin, antimycin A and dinitrophenol. In no case was any difference seen between the control animals and the thyroidectomized animals. Weight gain charts and the serum PBI determinations showed no differences between the two groups. This suggested that either the surgical procedure was ineffective or that the rats used were beyond the stage where thyroidectomy would have any major effect.

HD-AG 35

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#### Changes in Molecular Population During Development, Maturation, Aging, and Cellular Death

The general objectives of this work involve the description of the occurrence, properties, and changes in molecular species during various phases of the life cycle including development, aging, and cellular death. Investigations undertaken within this general framework include those to determine: (1) changes in parameters of oxidative phosphorylation with age; (2) lipid changes accompanying cell death, particularly as a result of surgically induced myocardial infarction; (3) changes in the synthesis and turnover of RNA with age; (4) changes with age in the pathway of biosynthesis of Coenzyme Q; (5) differences of tissues in individual transfer RNA species and specific amino acyl sRNA synthetases with regard to the possible involvement of these components in differentiation and

aging; (6) distribution of lipofuscin pigment in tissues of various animal species; and (7) the uptake of tritiated leucine in lipofuscin-containing tissues of senescent hamsters.

Measurements of labelled amino acid incorporation into sRNA and chromatography of acylated sRNAs on MAK columns are made using standard techniques. Unstained radioautograms are prepared from leucine-labelled tissues. Alternate viewing under fluorescence or phase illumination is used to correlate the positions of auto-fluorescent pigment particles and silver grains.

The parameters of oxidative phosphorylation were studied in mitochondria isolated from the livers, hearts and kidneys of young (3-6 month), mature adult (12 month), and senescent (24 month) rats. No age-associated decline could be seen in the P/O or the respiratory control ratio, indicating that there is no decline in the efficiency or integrity of mitochondrial energy transduction apparatus per se.

Work on lipid components of myocardial tissue after infarction was continued. The tissue was homogenized and fractionated by differential centrifugation, and the lipids of various subcellular fractions were extracted and examined with no major differences being found.

Preliminary study results suggested that in the mouse an age-associated decline in ribosomal RNA synthesis exists in liver and kidney.

Methodology for the isolation and estimation of the intermediates in the pathway of biosynthesis of the aromatic moiety of Coenzyme Q, utilizing techniques of thin-layer and gas-liquid chromatography, were worked out.

An alanyl sRNA species isolated chromatographically from rabbit tissues was found to react with unfractionated synthetase enzymes from rabbit liver and reticulocytes but not kidney. The extent of kidney deficiency in this enzyme was such that it appeared unlikely that kidney tissues can synthesize significant amounts of any protein coded with this particular alanyl-sRNA species, although other alanyl-sRNA species reacted well with kidney enzymes. A similar deficiency in synthetase activity towards a leucyl-sRNA species was found for rabbit reticulocytes, but it was not proven that this synthetase activity was completely absent from reticulocytes.

In related experiments, sRNA was acylated by a mixture of enzymes from 4 tissues and was deacylated by synthetase enzyme from individual tissues. The essential completeness of deacylation that occurred for all of the 7 amino acids and 4 tissues measured appeared to indicate that none of the tissues was deficient in any of the specific activating enzymes, either for major sRNA components or for the amino acids studied, which are present in other tissues. These results suggested that codon-activating differences may not be a general feature of differentiated cells.

A survey was made of unstained sections of various organs in the rat, mouse, hamster, guinea pig, tortoise, perch, and clam, and lipofuscin pigment was noted in all of these animals. Birth-dates were known in the rodent group and the senescent animals appeared to have more pigment than the young. In rat brain, quantitation of pigment by a grid reticule technique revealed that the hippocampus acquires pigment earlier and in greater amounts than does the cerebral cortex which is more involved than the caudate nucleus.

Thirty-two radioautograms were prepared following the injection and sacrifice of 3 senescent hamsters and comparison was begun of grain counts in the presence or absence of age pigment.

HD-AG 36

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#### Studies in the Relationship Between Proliferation, Regeneration, Differentiation, and Death of Cells in Vitro and in Vivo.

The various phases of this research are concerned with the relationship between cellular properties at various stages of the lifespan and the aging process.

Various tissue and cell culture techniques are adapted and employed, and appropriate specific radioactive tracer methods are used.

The growth span of embryonic chicken fibroblasts in vitro was found to be limited to 15-25 generations during a cultivation period of about 2 months. Varying the age of the donor embryo did not affect the growth span significantly. Measurements of metabolite flux (using labelled glycine, leucine and glucose) did not show gross differences between early and late passage cells; thus



diminishing the probability that large permeability changes occur during serial cultivation in vitro. This also indicated that permeability changes are not likely to be a major factor contributing to the cell deterioration observed. The possibility that metabolic potential of cells in culture is limited rather than division potential was tested through determination of the survival time and number of divisions undergone by cells maintained under agar in the stationary growth phase for various periods of time. The results suggested that total time in culture, not number of divisions, limits survival.

A descriptive histological and histochemical study of chick embryonic and human cell strains in culture was completed with a distinct increase in lipid content noted in late passage cells and marked changes in cell adhesion to glass substrate being observed.

Research on the effects of sera from human and bovine donors of various ages on proliferation of chick embryonic and human diploid fibroblasts as well as an heteroploid cell line (HeLa) was undertaken. An unexplained high incidence of general toxicity was obtained with human serum samples supplied by an outside laboratory. However, results with bovine sera showed a consistent decrease in cell proliferation when sera from older donors were used.

In another phase of this project, improved techniques were used to cultivate Tribolium embryonic cells in vitro. These cells from 5-to-6-day-old embryos of this insect species were found to grow in Grace's medium supplemented with NaCl and 0.5% lobster hemolymph. It was found that the growing cells form into a network of fibroblast-like cells and epithelioid cells with the fibroblast-like cells disappearing in about two weeks. On the other hand, the epithelioid cells were maintainable in cultures for over three months with regular changing of the medium.

Study of the ability to synthesize RNA and DNA in vitro in bone marrow cells of young donor rats versus older donor rats indicated there were no differences in this ability. However, there might be a difference in the precursor pools.

Early work on RNA synthesis in vivo in mice indicated that ribosomal and possibly transfer RNA may be synthesized at a lower rate in kidneys and liver of old animals than in those of the young. Also, an age difference in the RNA and DNA content of kidneys was found with old kidneys having less RNA and more DNA than young kidneys. No differences were noted in liver content.



HD-AG 37

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#### Studies of the Comparative Physiology of Aging

This study is designed to extend previous light microscope studies of morphological and histochemical changes during the aging and regression of hydranths to the ultrastructural level, and to determine the role of lysosomes in the degradation of each hydranth cell type. Also sought is a description of histochemical changes in the female reproductive system of the flour beetle during the aging process and the correlation of these changes with the functional capacity of these organs. In addition, studies on the histochemical changes of the rat pituitary during the aging process are conducted.

Methods used include histochemical procedures, microchemical assay for enzymes, and electron microscopy.

The ultrastructure of young (2-day-old) Campanularia flexuosa hydranths has been described showing that the animal is composed of two epithelia, ectoderm and gastroderm, separated by the acellular middle lamella, the mesoglea. Cell types in the ectoderm of the tentacles and body are: epitheliomuscular; nematocyte, secretory type 1, secretory type 2, and interstitial. Gastrodermal cell types are: mucous, zymogen, digestive, and secretory type 2. The subcellular morphology of each cell type has been characterized and compared to similar cell types in other animals where they have been described.

Histochemical studies of 6-month-old, 12-month-old and 24-month-old rat pituitary glands have revealed certain age-related cytochemical and histological changes.

HD-AG 38

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#### Fractionization of Aging Connective Tissue

The objective of this study is to find out how collagen is serially depleted from a fibrous matrix under conditions that favor solubilization, and to find either the existence or non-existence of a sharp demarcation between labile and refractory collagen fragments. Secondly, this project is an effort to determine the number of components obtained by a standard extraction procedure, and the molecular weight of each such component. The information

obtained could then be used to construct a serial elution concentration-profile for collagen fibers. Also, a nomogram of this type could be used as a rapid way to chronologically follow polymerization of collagen fibers during growth-development, aging, and pharmacologic control.

Samples of fetal calf skin are extracted with hot ethanol to remove fat, then soaked in acetone, evacuated to constant weight, then stored in a dessicator. One hundred milligram samples are serially extracted with 3 mls. of water for 30 minutes at temperatures of 40, 50, 60, 65, and 70°C. The optical density of 250 millimicrons and that of the tyrosine reaction product (Folin-Ciocalteu) are used to follow the serial extraction. Residual protein is then autoclaved serially. Finally, a series of autoclaved digests are made on samples which have not been serially extracted.

The concentration of substances eluted serially from fetal calf skin (gestational age of 100-250 days) decreased non-linearly with repeated equilibrium of residue with fresh solvent.

The total quantity of protein in the sample decreased exponentially with increasing gestational age. Rate of decline of the sum of serial soluble components was less than that of the insoluble residue.

Serial elution of components from melted rat tail tendon was reported previously. Since that time, these extracts have been separated by electrophoresis on acrylamide gel thus revealing the presence of more than the three components usually seen for melted collagen. It was found that extensive thermal degradation of purified collagen does yield extra fragments by column chromatography.

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Elasticity of Aging Connective Tissue:  
Cohesion of Collagen Fibers

This project is an effort to determine whether or not changes observed in fiber diameter at the molecular level (electron microscopic data) exert an influence on fiber cohesion at the macroscopic level, and to find out if inter-fiber cohesion depends upon animal age and endocrine factors as regulating agents.

Rat tail tendons are separated and washed in saline for five minutes followed by rinsing in water. Tendons are lifted out individually and dried. The ratio of weight/length is determined and called  $W_0$  (mgs./cm.) and this is used as an index of cross sectional mass since it equals cross sectional area density. Tests have shown that on melted tendon force-time or length-time data are influenced by aging and that the time required to depolymerize tendon to the point of rupture is sensitive to age. This time-to-break ( $T_B$ ) is measured by attaching a 10-gram weight to a single tendon folded into a loop and suspended from a micro switch. Five molar urea at 40°C is added to the test tube surrounding the tendon and maintained at constant temperature. The clock stops automatically when the tendon ruptures. Statistical evaluation of data is done by computing mean ( $\bar{W}_0$  and  $\bar{T}_B$ ) and standard deviation (SD  $W_0$  and SDTB) values for at least 10 tendons from each animal. Manor Farm strain male and female rats are used to study aging from 1 to 12 months; supplemental data are obtained from Wistar rats to 24 months. Additionally, tendons are obtained from a series of endocrine disturbances induced in male Carworth Farms rats at the age of 4 weeks and examined 2 weeks later.

Results showed that the cohesion of tendon fibers, represented by  $W_0$ , the cross sectional mass, increased during growth from 1 to 12 months. Elevation of  $W_0$  with body weight included an initial phase of linear growth (0 to 100 grams) followed by a period of rapid exponential increase. However, from 12 to 24 months of age,  $W_0$  increased at a much slower and nearly linear rate.

Tendons from rats which had undergone hypophysectomy, thyroidectomy and adrenalectomy were tested and it was noted that  $\bar{W}_0$  and SD  $W_0$  were linearly inter-related. To check on this dependence, data were obtained for the dependence of both parameters on body weight and it was shown that following endocrine disturbances in young animals, there was a retardation of growth rate in the hypophysectomized animal. Progressively less inhibition was produced by the other procedures.  $W_0$  changed with body weight of disturbed animals in the same dependence on weight as observed with natural aging (growth from 1 to 12 months), and SD  $W_0$  followed in similar fashion. This suggested that animal age during growth is not the fundamental parameter to which  $W_0$  and SD  $W_0$  are related. Instead it is the endocrine (or other) systems which regulate overall protein synthesis and depletion.

The time-to-break ( $T_B$ ) was found to be a function of  $W_0$ , but did not show as great a dependence as it had on age and endocrine disturbance.  $T_B$  increased exponentially with aging in the 12 to 24 month interval while mean  $W_0$  only increased slightly and linearly.

These findings suggested that deposition and cohesion of collagen fibers are regulated by hormonal agents (pituitary) in a linear proportion to total body mass.

HD-AG 40  
Harry R. Elden  
Gerontology Research Center, NICHD  
Baltimore, Maryland

#### Hydration of Aging Connective Tissue

This project's objective is to ascertain whether or not hydration of collagen produces a continuum of binding sites for absorbed molecules, or whether there are specific sites with varying affinities for probing molecules.

Preparations of rat dermis and fetal calf skins are used. Samples are dehydrated over sulfuric acid and then placed in methanol, ethanol and iso-propanol in a separate series of equilibrations. Sample weight is measured until constant uptake of solvent is achieved. This requires 2 to 3 weeks in some cases. All equilibrations are done at 20°C in a constant temperature water bath.

This study showed that alcohols react with hydrated collagen, but the extent of reaction is determined by the collagen's water content and the specific alcohol used. Methanol sorbs onto collagen at the same extent at all water contents of collagen. Iso-propanol would not sorb onto dry collagen but did react with hydrated collagen. The extent of this reaction was related linearly to the relative humidity of water vapor in which the collagen was equilibrated. Ethanol and n-propanol were proportionately interposed between the affinities of methanol and iso-propanol. These findings indicated that binding sites of hydrated collagen are heterogeneous and can be differentiated by physical properties of probing molecules.

A study of fetal growth in calf skin and post-fetal growth in rat skin using methanol and iso-propanol showed that there was no difference in the methanol uptake over a relative humidity range of 0.15 to 1.00 for rat dermis (5 weeks to 12 months). Earlier reports showed that uptake of water vapor over this range of relative humidity was not influenced by age. Similarly, it was found that absorption of water from the liquid state by aging rat dermis was independent of age. Water content of fetal calf skin (110 to 240 days gestation) did not change with age, but the alcohol taken up (methanol and iso-propanol) showed a tendency to increase during this period. Water content of samples equilibrated over water and



40% sulfuric acid, however, did not change. It was concluded that the interior structure of collagen in rapidly growing skin (fetal age) has greater heterogeneity than that of post-fetal skin. Also, differential binding by alcohols was found to be greater in fetal skin than in rat skin, and more sensitive to heterogeneity than water binding.

HD-AG 41

Joseph Avruch

Gerontology Research Center, NICHD

Baltimore, Maryland

#### Efflux of Free Fatty Acids from Isolated Adipose Tissue Cells

This investigation is aimed at examining outward flux of free fatty acids (FFA) by prelabeling isolated fat cells with tritiated palmitic acid and following the distribution of radioactivity between the intracellular and extracellular FFA fractions under a variety of conditions.

Isolated fat cells are prepared by the Rodbell method. During incubation of the adipose tissue with collagenase, the cells are simultaneously incubating with  $H^3$ -palmitate-albumin. At the end of an hour the cells are washed several times in albumin-free medium and resuspended in media containing fatty acid-poor albumin, and the appropriate experimental and control additions. At intervals, after further incubation, control and experimental samples are taken. Cells are separated from medium either by filtration or gentle centrifugation and washing of cells in albumin-free media. Glycerol in the medium is measured enzymatically. FFA in the medium are extracted substituting hexane for heptane. An aliquot is used for liquid scintillation counting. Extracellular FFA is obtained by extracting the lipids. The extracts are pooled and taken to dryness. Tissue residue is washed twice with cold 5% TCA. DNA is estimated by a modification of the Kissane and Robbins method. Lipid extract is redissolved in hexane and fatty acids (and some phospholipid and lower glycerides) are extracted by successive aqueous alkaline washes, are pooled, acidified, and washed again with hexane. Hexane extract is subjected then to thin-layer chromatography; fatty acid spot is scraped off, eluted and counted in a liquid scintillation counter.

Validation of the method for this study has been made and various fractions have been recovered.



HD-AG 42  
William B. Weglicki  
Gerontology Research Center, NICHD  
Baltimore, Maryland

Distribution and Metabolic Functions of Vitamin E in Young and Aged Rat Hearts: Effects of Vitamin E Deficiency on Biochemical and Pathophysiological Parameters

The purpose of this work is to determine levels of vitamin E in heart muscle of young and aged rats; to localize distribution of vitamin E in heart muscle (beef heart model), and to establish correlations between changes in biochemical, histopathological (myocardial age pigment) and physiological parameters (perfused rat heart preparation) of the vitamin E deficient rat.

Two models are studied: rat myocardium and beef heart (mitochondria). Groups of rats weaned at 3 weeks of age are given vitamin E deficient and sufficient diets. Tail blood samples are tested for red cell hemolysis at two week intervals. When deficient animals manifest greater than 90% hemolysis, they are sacrificed and their myocardial mitochondria studied for enzymatic activity. Enzymes assayed are DPNH cytochrome c reductase and succinate cytochrome c reductase. None of the control animals demonstrate hemolysis by dialuric acid. When a more precise determination of vitamin E deficiency of the myocardium is necessary than that provided by the indirect red cell hemolysis technique, the myocardial vitamin E levels are determined through a technique employing homogenization, centrifugation, extraction of tissue lipids in organic solvents, saponization, removal of sterols, thin layer chromatography, and identification of vitamin E by spectroscopy, spectrofluorometry and gas-liquid chromatography. Heavy beef heart mitochondria and rat myocardial whole homogenates are prepared and vitamin E levels measured.

So far no differences in the enzyme activities studied have been noted in vitamin E deficient rats when compared to the control group. Red blood cell deficiency of vitamin E did not substantiate the assumption that the myocardium was deficient to the same degree, and that only direct measurement of myocardial mitochondrial vitamin E content could substantiate the conclusion reached concerning myocardial enzyme activity.

HD-AG 43  
Harry R. Elden  
Gerontology Research Center, NICHD  
Baltimore, Maryland

### Endocrine Regulation of Connective Tissue Systems

This project is an attempt to devise a way in which the dynamicity of connective tissue can be measured and compared with that of other systems. Through measurements of the biological dynamicity of physiological organ systems as a function of age it may be possible to answer the following questions: Does the accumulation of refractile insoluble fibers, constituting a type of progressive decline in biological dynamicity with aging, proceed independently of other physiological systems, or does it occur via automatic decline of a superior control system to which connective tissue is attached? And, does the change in connective tissue dynamicity lead to reduction in sensitivity of other systems such as those that influence a host's resistance to pathological degeneration and eventual death?

A large number of weanling female rats are acquired and maintained by the animal supplier until needed. At various time intervals of 6, 8, 11, 16, 24, and 36 weeks after acquisition, groups of animals are subdivided into sub-groups, with one group serving as a surgical control and the others being hypophysectomy (HX), thyroidectomy (TX) and adrenalectomy (ADX). These latter surgically induced endocrine disturbances are repeated for groups selected at the above mentioned ages. Surgery is done by the supplier with shipment a week later. Animals shipped are maintained in the laboratory on appropriate diets. At closely spaced intervals in serial progression, animals are sacrificed from the four sub-groups of control (c), HX, TX, and ADX. Major organs such as heart, liver, kidneys, uterus, ovary, and adrenals are carefully dissected out and weighed.

The investigators found that the growth rate of HX animals was reduced below that of the controls. TX and ADX had only a slight influence on growth rate giving an approximate measurement of the contribution of surgical trauma alone. Growth rate of HX animals was shown to be related to the age at which endocrine disturbance was produced. Six week animals showed a positive weight gain following HX, but growth rates of the other age groups were considerably less relative to the control animals. Organ weights of HX animals were significantly lower than for controls or the TX and ADX animals.

The velocity of weight gain during growth and velocity of reaction to endocrine disturbance appeared to progressively and continuously decline with age during growth-development. There was a slight response noted in animals to thyroidectomy, but no response with adrenalectomy. This indicated that the pituitary gland operates partially through the thyroid but not the adrenals in the control system. It operates mainly in a by-pass of the thyroid, either directly or by other mediation, since its absence (HX) causes a far greater disturbance than is achieved by TX.

ATOMIC ENERGY COMMISSION CONTRACTS



# ATOMIC ENERGY COMMISSION CONTRACTS

<u>GRANT NUMBER</u>	<u>INVESTIGATOR, PROJECT TITLE</u>
AT(04-1)-GEN-12	Leslie R. Bennett Laboratory of Nuclear Medicine and Radiation Biology University of California Los Angeles, California Late Effects, Radiobiology
AT(04-1)-GEN-12	James F. Mead Laboratory of Nuclear Medicine and Radiation Biology University of California Los Angeles, California Effect of Aging and Irradiation of the Brain Lipids
AT(11-1)34, Project 41	David A. Wood and Paul H. Guttman San Francisco Medical Center University of California San Francisco, California Study of the Kidney in Aging and X-Irradiated Animals with Particular Reference to the Role of Immunity in the Pathogenesis of Late Effects of X-Ray
AT(11-1)1474	Kenneth Brizzee University of Utah Salt Lake City, Utah Long Term Effects of Prenatal X-Irradiation on Cerebral Cortex
AT(30-1)1243	Brown Dobyns Case-Western Reserve University Cleveland, Ohio A Study of the Physiological Function and Histological Changes of Thyroids Irradiated with Radioactive Iodine
AT(30-1)2071	Robert L. Brent Jefferson Medical College Philadelphia, Pennsylvania The Effect of Embryonic Irradiation on Adult Life Expectancy and Adult Pathology in Mice and Rats

AT(30-1)3314	John Yuhas The Jackson Laboratory Bar Harbor, Maine Genetic Control of Aging and Radiation-Induced Life-Shortening in Mice
AT(30-1)3394	Thomas Mancuso University of Pittsburg Hanford Occupational Health Fdn. Union Carbide Corporation Social Security Administration Washington, D.C. Study of Mortality Patterns in AEC Contractor Installations in Terms of Different Environmental Exposures
AT(30-1)3518	Verner J. Wulff Masonic Foundation for Medical Research and Human Welfare Utica, New York A Study of the Effects of Age and Ionizing Radiation on Nucleic Acid Metabolism and Protein Synthesis in Visceral and Central Nervous System Tissues
AT(30-2)-GEN-16	Howard Curtis Brookhaven National Laboratory Upton, New York Biological Research Effects of Radiation on Aging in Mice
AT(45-1)581	Robert Koler and Demetrios Rigas Univ. of Oregon Medical School Corvallis, Oregon Studies of Genetic Alterations in Human Cells and Molecules and Factors Influencing Them
W-31-109-ENG-38	George A. Sacher, Samuel Leshner, Michael Fry, and G. Mark Kollmorgen Argonne National Laboratory Argonne, Illinois External Radiation Toxicity Effects on Cells and Physiological Mechanisms
W-31-109-ENG-38	George A. Sacher Argonne National Laboratory Argonne, Illinois Theoretical Biology

W-7401-ENG-49

Robert C. Baxter  
Atomic Energy Project  
University of Rochester  
Rochester, New York  
Radiobiologic Studies  
with Drosophila

W-7401-ENG-49

George Casarett and Hugh Eddy  
Atomic Energy Project  
University of Rochester  
Rochester, New York  
Pathologic Mechanisms of  
Permanent and Delayed  
Radiation Effects

W-7401-ENG-49

George W. Casarett and Hugh Eddy  
Atomic Energy Project  
University of Rochester  
Rochester, New York  
Effects of X-Irradiation on  
Spermatogenesis in Dogs

W-7405-ENG-26

Takashi Makinodan  
Oak Ridge National Laboratory  
Oak Ridge, Tennessee  
Growth and Senescence of  
the Immune Mechanism

W-7405-ENG-26

Arthur Upton  
Oak Ridge National Laboratory  
Oak Ridge, Tennessee  
Long-Term Effects of Irradiation

W-7405-ENG-48

Patricia W. Durbin  
Lawrence Radiation Laboratory  
Berkeley, California  
Growth and Senescence of the  
Soft Tissues and Skeleton  
in the Normally Aging Female Rat

AT(04-1)-GEN-12  
Leslie R. Bennett  
Laboratory of Nuclear Medicine  
and Radiation Biology  
University of California  
Los Angeles, California

#### Late Effects, Radiobiology

Studies revolve around the basic problem of the nature of the life shortening effect of irradiation. This is approached by life span studies of the incidence of disease and age specific organ function in the locally and totally irradiated animal. The late effects of selective irradiation of the liver, kidney, and extremities are investigated. Longevity is the variable of prime interest in this program and is used as the end point measure of radiation effect. This approach requires three to four years for each experiment. A search is conducted for physiologic measures that correlate well with ultimate longevity and, hopefully, will provide an earlier measure of delayed radiation effects. The demonstration in this laboratory that at least one metabolic aberration, i.e., enhanced salt toxicity, can be measured in post-irradiated rats during the disease-free latent period is a stimulus to continue the effort to define the late effects syndrome and longevity in biochemical and physiologic terms. Carbon tetrachloride and other forms of biochemical stress are studied as a means of bringing out latent injury.

AT(04-1)-GEN-12  
James F. Mead  
Laboratory of Nuclear Medicine  
and Radiation Biology  
University of California  
Los Angeles, California

#### Effect of Aging and Irradiation of the Brain Lipids

The lipids of aging and irradiated human and rat brains are separated by column and thin-layer chromatography into their components and these are converted, where appropriate, into the methyl esters of their component fatty acids, which are analyzed by gas-liquid chromatography.

AT(11-1)34, Project 41  
David A. Wood and Paul H. Guttman  
San Francisco Medical Center  
University of California  
San Francisco, California

Study of the Kidney in Aging and X-Irradiated Animals  
with Particular Reference to the Role of Immunity  
in the Pathogenesis of Late Effects of X-Ray

One phase of the project is a continuation of previous work on aging with particular reference to progressive alteration of the intercapillary tissues of the glomerulus, a process termed intercapillary clomerulosclerosis. The present study will give us additional information on the finer histological alterations (electronmicroscope) of the renal glomerulus of the mouse associated with aging and following irradiation.

Balb/c mice were x-irradiated at age of 40 to 50 days with doses ranging from 350 to 2100 R. Sacrifices are scheduled at 150, 300, 600, and 1000 days. Studies will be made under phase microscope and with the electron microscope using special stains and fixative in effort to study and preserve the intercellular basement membranes and ground substance. Similar studies are being done in the kidneys of beagle hounds from the long-term experiments at Davis, California. At present mice subjected to x-irradiation 150 days previously are being studies with methods outlined above. The results will be compared with similar studies conducted at 300 days, 600 days and 1000 days post-irradiation.

The second phase parallels the foregoing phase, and studies the capillaries in the retina and heart of mice with particular reference to the structural changes in the endothelium, basement membranes and pericyte with age and late effects of irradiation. The purposes are to determine the following: (1) are the changes in the intercapillary cells of the glomerulus a part of a generalized process which effects related vascular cells -- the pericyte -- in other organs and tissues? (2) to compare the late effects of graded doses of x-ray on the endothelial cells and the pericyte.

Balb/c mice of age 40 to 50 days have been irradiated to upper half of body with x-rays ranging from 350 R to 2100 R. Sacrifices are to be made at 150, 300, 600 and 1000 days. The ultrastructure of the capillaries will be studied using an RCA electron microscope. Sections will be fixed in gluteraldehyde and osmic tetroxide and embedded in araldite. Special pre-staining and post-staining technics will be used for study of cells in intercellular structures.



AT(11-1)1474  
Kenneth Brizzee  
University of Utah  
Salt Lake City, Utah

Long Term Effects of Prenatal X-Irradiation on Cerebral Cortex

Studies on the effects of prenatal whole body x-irradiation (90-130R) will be continued on cerebral cortex and subdivision of the brain in adult rats at progressively older age levels. Gross parameters to be investigated include: total brain weight, weight of major structural subdivisions of brain, dimensions of cerebral hemispheres, ratios of brain weight to body weight, and the ratios of the weights and dimensions of the major parts of the brain to each other. Micro parameters will include a qualitative description of cytologic and vascular changes in cerebral cortex employing a 2-stage perfusion fixation method. Quantitative studies will include a determination at 20 successive cortical depth level and in individual cytoarchitectonic cortical laminae of cell packing density and glia/neuron by capillaries and by larger vessels (vascular volume). Branching patterns, orientation and territory occupied by dendrites will be studied in Golgi-Cox stained tissues. Autoradiographic studies on glial cell multiplication in adult cerebral cortex in irradiated and control rats will be conducted at progressively older age levels by determining the number or proportion of H-3 thymidine labelled glial cells in dorsolateral wall of lateral ventricle and in cerebral cortex.

AT(30-1)1243  
Brown Dobyns  
Case-Western Reserve University  
Cleveland, Ohio

A Study of the Physiological Function and Histological Changes of Thyroids Irradiated with Radioactive Iodine

This long-term project is concerned with the investigation of radiation effects on animal and human thyroids. Detailed kinetic data are being collected on selected patients given  $I^{131}$  to correct hyperthyroidism. These data are ultimately related to clinical changes. In these selected human subjects the emphasis is not only on the clinical and physiologic changes, but also on the ultimate morphologic changes when there are opportunities to obtain tissue.

A study is in progress to determine the incidence of anomalies in circulating leucocytes of hyperthyroid patients treated with  $I^{131}$  as was done in the past where large doses had been used in cancer of the thyroid.

Quantitative measurements of DNA in a series of human radiated tissue are being made. The exaggeration of bizarre nuclear forms, demonstrated by the administration of antithyroid drugs in  $^{131}\text{I}$  treated animals, is now being looked for in man.

The technique of administering tritiated thymidine followed by radioautography is being used to study the process of DNA synthesis in rat thyroids during the induction of neoplasms following  $^{131}\text{I}$ . This technique is also being used to study the lifespan and normal replication rate of thyroid cells in young and old animals so that comparisons can be made in radiated cells.

AT(30-1)2071  
Robert L. Brent  
Jefferson Medical College  
Philadelphia, Pennsylvania

The Effect of Embryonic Irradiation on  
Adult Life Expectancy and Adult Pathology in Mice and Rats

The purpose of this research project is to determine whether the mammalian embryo is more sensitive to the tumorigenic and life-shortening effects of x-irradiation than is the same organism when irradiated after parturition (mouse). Secondly, the extent and persistence of radiation induced biochemical and physiological alterations will be evaluated in the adult animal following their production at specific times during gestation in the rat fetus. Thirdly, the relative importance of ovarian irradiation, uterine irradiation, maternal irradiation and zygote irradiation are being investigated as to their contribution to embryopathology, observed in the embryo and fetus.

Low dose (30-90r) embryonic irradiation has not altered the lifespan of irradiated embryos when irradiated on 7, 8, 12 and 16th day of pregnancy, although postpartum growth retardation was readily produced. Irradiation of the rat zygote and oviduct on the first day of gestation with 150r resulted in a 70% mortality and no increase in congenital malformations. Irradiation of the ovary and uterus did not contribute to embryonic mortality. On this day of gestation, no maternal effect was observed except for the possibility of alterations in the irradiated oviduct.

AT(30-1)3314  
John Yuhas  
The Jackson Laboratory  
Bar Harbor, Maine

Genetic Control of Aging and Radiation-Induced Life-Shortening in Mice

The research supported under this contract is designed to provide an indirect test of the somatic mutation theory of aging and radiation-induced life shortening. We have postulated that the stability

of the genetic material or its resistance to mutation is dependent upon genotype (strain) and is itself genetically controlled. If this postulate is correct and if somatic mutations are responsible for aging and radiation-induced life shortening, then it follows that: (a) different genotypes (strains) should age at different rates, (b) inheritance of the rate of aging should be demonstrable by standard genetic techniques, (c) the extent of radiation-induced life shortening should vary with strains, (d) there should be direct evidence of strain differences in the rate of increase in mutations with age, and (e) the yield of mutations following a standard radiation exposure should differ as a function of mouse strain.

To test these five inferences, populations of mice of both sexes from four inbred strains and two F<sub>1</sub> hybrid generations have been exposed to one of three levels of radiation exposure (zero, 250, or 450 R) and set aside for determination of their complete life tables and pathology at death. The incidence of gross chromosomal aberrations in two tissues as a function of age or following a standard x-ray exposure is being determined in the same strains and hybrids. The incidence of lethal mutations in spermatozoa as a function of age of the male parent or following a standard x-ray exposure is also being estimated in these strains and hybrids.

AT(30-1)3394  
Thomas Mancuso  
University of Pittsburg  
Hanford Occupational Health Foundation  
Union Carbide Corporation  
Social Security Administration  
Washington, D.C., and Pittsburgh, Pennsylvania

Study of Mortality Patterns in AEC Contractor Installations  
in Terms of Different Environmental Exposures

The objective remains the same: to carry out test runs of cohort employee populations of selected AEC Contractor installations, in order to assess the feasibility of using personnel, employment, medical and radiation records in establishing the relationships, if any, between mortality patterns and levels of radiation exposure. The reason for the study is the absence of comparable information pertaining to human populations exposed to recorded low levels of radiation over long periods of time. The general procedure initially established will be continued: to establish a series of cohorts at each facility, including those employed as well as those separated for each year; to trace these individuals through Social Security records to determine those who have died and their place and date of death; to obtain death certificates to establish the mortality pattern; and to evaluate the relationships, if any, of the mortality patterns of these cohorts with radiation, length of employment, and other environmental data. The following AEC Contractor facilities were selected

for test runs: the three Oak Ridge facilities, Hanford, and several small feed materials plants, providing populations with the longest intervals of operation. At the end of the second year of this five-year study, all available radiation exposure records will have been compiled or abstracted by annual readings and recorded on ADP equipment for Oak Ridge and Hanford. Also, the Hanford Occupational Health Foundation will have completed recording the necessary personal identification and sibling data from 1944 through 1966, and a study of medical x-ray exposures received by employees from 1946-1956. During the third project year, the recording of personal data on Oak Ridge employees and feed materials centers will be intensified, other phases of the project will be continued, and the searching for deaths through the Social Security Administration will begin.

AT(30-1)3518

Verner J. Wulff

Masonic Foundation for Medical

Research and Human Welfare

Utica, New York

A Study of the Effects of Age and Ionizing Radiation  
on Nucleic Acid Metabolism and Protein Synthesis  
in Visceral and Central Nervous System Tissues

The work proposed deals with the investigation of age-associated changes in 1) mitotic activity and DNA turnover, 2) molecular and cellular heterogeneity, 3) the genome, 4) RNA metabolism in the Central Nervous System and 5) the effect of circadian rhythm and x-irradiation on RNA metabolism in liver. This work will be performed with rat tissues and will involve the following techniques: 1) The measurement of mitotic index and  $H^3$ -thymidine incorporation in rats of 3 ages, using colchicine to arrest mitosis, including experiments to assess the effect of colchicine and colcemid and the effect of pretreatment with cold thymidine on the incorporation of  $H^3$ -thymidine into DNA of rat liver; 2) The measurement of changes in staining reactions for hydrolytic enzymes and changes in the soluble protein populations in rat liver; 3) The measurement of template activity, hydrogen exchange and fractionation, using methylated albuminkieselguhr chromatography, of chromatin and DNA from liver of young and old rats; 4) The measurement of RNA metabolism in diced liver preparations, brain slices and metabolically active liver nuclei by measuring the rate of incorporation of labeled precursors.

AT(30-2)GEN-16

Howard Curtis

Brookhaven National Laboratory

Upton, New York

Biological Research Effects of Radiation on Aging in Mice

The life-shortening effect of radiation in animals, known as "radiation-induced aging," is a well-known but poorly understood



phenomenon. Its relationship to natural aging is obscure. This program attempts to find similarities and differences between the two forms of aging and to understand both phenomena in terms of basic biology.

One theory proposes that aging is the result of an accumulation of chromosome aberrations (mutations) in the cells of somatic tissues. The theory is being tested in mice. The tissue under study is liver. To obtain the dividing cells in which chromosome aberrations are detectable, the tissue is induced to undergo regeneration. The following has been established: (1) In normal mice the number of aberrations (mutations) in liver increases steadily with age. (2) Following a single dose of x-rays, the aberration frequency promptly increases to a very high level from which it slowly declines with time. (3) Another mutagenic agent, nitrogen mustard, does not increase the mutations in non-dividing cells but does in dividing cells, and it also does not shorten life-span. This indicates that the non-dividing cells of the body are primarily responsible for aging. (4) Chronic gamma-ray irradiation is only about 25% as effective in causing both life shortening and chromosome aberrations as is acute irradiation. (5) Conversely, chronic neutron irradiation is just as effective in causing both life shortening and chromosome aberrations as is acute neutron irradiation. (6) Single doses of neutrons are about twice as effective in producing both life shortening and chromosome aberrations as are single equivalent doses of x-rays. (7) A short-lived strain of mice develops chromosome aberrations at a much faster rate than a long-lived strain. (8) Chromosome breaks heal, sometimes over a period of months. The healing process is as important as the factors leading to chromosome instability in the aging process. These findings strongly support the somatic mutation theory of aging and give a firm cellular basis for believing that radiation accelerates the aging process.

In addition to the study of the theory, the relationship between organ function and aging, both natural and radiation-induced, is under examination. The kidney has been most extensively studied so far. Function in this tissue appears to be remarkably resistant to both aging and radiation.

AT(45-1)581  
Robert Koler and Demetrios Rigas  
University of Oregon Medical School  
Corvallis, Oregon

Studies of Genetic Alterations in Human Cells and Molecules  
and Factors Influencing Them

The objective of this project is the study of: (1) The mechanism of biochemical and biophysical processes as they relate to the structure and function of proteins, (2) The aging as affected by post-synthetic structural changes of macromolecules (macromolecular aging),



and (3) The mechanism of controlling and initiating cell division. Such studies are needed because of observations that: I) Genetic diseases affecting the primary or quaternary structure of protein molecules often result in marked changes of the biological activity of the involved molecules. Knowledge of the relationship of structure to function of such molecules will help the elucidation of the mechanism of their action and their significance in the clinical manifestations of disease. II) Aging of animals and of erythrocytes is accompanied by changes in the structure of collagen and of hemoglobin respectively. The importance of such changes needs to be evaluated. III) Cell differentiation and malignancy are processes involving the control and initiation of cell division which are still unknown. Studies on the relationship of structure to function of the phytohemagglutinin, which we have isolated in homogeneous form, will be carried out by completely characterizing its molecular structure and subsequent studies of the effect of chemical modifications of the molecule on its mitogenic and hemagglutinating activities. The in vivo aging of the human hemoglobin A, which we have observed and which results in increased oxygen affinity, and of the abnormal hemoglobin H, which causes the destruction of the erythrocytes, will be investigated by preparing hemoglobin from young and old erythrocytes separated by ultracentrifugation, completely characterizing and comparing them structurally, in terms of their dissociation into subunits, and of their interaction with oxygen under various conditions and chemical modifications. The transformation of non-dividing lymphocytes into dividing cells by phytohemagglutinin will be followed with electronmicroscopic and metabolic studies.

W-31-109-ENG-38

George A. Sacher, Samuel Leshner,  
Michael Fry, and G. Mark Kollmorgen  
Argonne National Laboratory  
Argonne, Illinois

External Radiation Toxicity Effects  
on Cells and Physiological Mechanisms

A. Acute Whole-Body Irradiation Effects on Cell in Rodent Intestine

Damage and recovery in the cell-renewal system of the mouse small intestine are studied after single radiation exposures to obtain in vivo data comparable to information obtained from in vitro cell culture experiments. These studies utilize high-resolution autoradiography, after the injection of tritiated thymidine (a DNA precursor), and a newly developed cryptsquash technique that makes it possible to evaluate the effects of irradiation on the ability of proliferative crypt cell populations to synthesize DNA and divide in a highly quantitative manner.

## B. The Study of the Effects of Aging and Irradiation on the Tensile Strength and Elasticity of Tissues

As part of the Janus program, one of the major problems is to find suitable methods to obtain quantitative data for the changes occurring in tissues of a large number of animals with both aging, and acute and chronic ionizing irradiation. Changes in the physical characteristics of tissues have not been widely used to detect irradiation effects. Investigations will be carried out by using an Instron materials-testing instrument that will include the changes in tensile strength of the rat vibrissae and the compression characteristics of lens of the eye. It is hoped that the latter will give information about the pattern of changes that occur prior to cataract formation. A number of tissues are suitable for investigating aging changes; when suitable sampling techniques for these tissues are worked out, they will be included in the program.

## C. Cell Proliferation and the Effects of Irradiation

It is necessary to select suitable cell systems to investigate certain aspects of normal cell proliferation in relation to the effects of both aging and irradiation. First, we need a cell-renewal system in which the kinetics are different from the rapid-renewal systems such as gut and marrow. The following features are also considered desirable: (1) the basic indices of proliferation (labeling and mitotic index) can be determined for the total population and not only for the proliferative population; (2) chromosome preparations can be made easily; and (3) the tissue can be cultured. All these prerequisites are met in the selection of the corneal epithelium. Other advantages of the cornea are: (1) Alteration of the  $O_2$  tension in a controlled manner is more feasible in the cornea than in most other in vivo proliferative systems, and therefore, it is a suitable system to study the effects of  $O_2$  per se as well as the oxygen effect in irradiation; (2) The cornea, in some respects, is similar to a cell culture in vivo.

Second we need a cell system in which renewal occurs at a very slow rate but that can be readily stimulated into a high rate of proliferation. Such a system can be used to study both residual damage and the induction of rapid proliferation. Liver tissue is most commonly used for such studies, but, because of its complexity, it has certain disadvantages so we investigated the possibility of using two epithelia for this study -- the simple epithelium of the gallbladder and the transitional epithelium of the urinary tract. Both tissues have slow cell renewal rates, but they can be stimulated into rapid proliferation.

In the case of the gallbladder epithelium, proliferation is stimulated by choleretics and the urinary tract epithelium is stimulated by distension of the tract from a blockage of either the urethra or the ureter. These tissues can be studied autoradiographically in either sections or squash preparations and chromosome preparations can be obtained from them. We have found that scintillation-counting methods can be used in studies of the rat bladder. These tissues should also be suitable for the investigation of the relationship of proliferative activity to the induction of tumors by irradiation.

#### D. Radiation Effects on Cells and Physiological Mechanisms

Experiments in progress are designed to study the duration and variability of the phases of the generation cycle in both normal and treated (drug and/or irradiation) populations of mammalian cells grown in vitro. In addition, the effects of drugs and/or irradiation on the birth, morbidity, secretory, and cell death rates are being measured to provide a basis for a model of the effects of long-term radiation exposure on population structure.

The neoplastic mast cell (P815Y) was chosen as a unique cell line on which to measure radiation effects because both its functional differentiation and its proliferative processes are maintained in vitro. However, this line does not consistently lend itself to isotope-labeling and autoradiographic procedures necessary to measure the flow of cells around the mitotic cycle. The line has been retained to measure secretory activity (histamine and 5-hydroxytryptamine) as it relates to radiation damage and recovery following treatment.

Several additional lines have been acquired to study disturbances to the movement of cells around the mitotic cycle after drug and/or radiation exposure. One of these lines is a clone (2071) of Earle's L cells that has been adapted to protein-free, chemically-defined media. The morphological characteristics and growth patterns of this line are such that individual cells can be followed on a time basis by serial photography without disturbance to the culture. Another line being studied is the Chinese Hamster (Cricetulus griseus) lung cell because its diploid karyotype (22 chromosomes) is maintained after transformation. Both lines lend themselves well to autoradiographic techniques and tend toward homogeneity in response; the former because fluctuations in serum characteristics are not operative, the latter because the karyotype is discrete with little variation around the modal chromosome number.

### Theoretical Biology

#### A. Theory of Aging, Mortality and Radiation Injury

The theoretical study of stochastic fluctuations in basic physiological functions, and of their contribution as causes of mortality in biological populations, is basic to the experimental work performed in radiation toxicity. In addition to this main line of inquiry, four related programs are carried under the same heading: a) theoretical study of radiation injury and recovery, b) determination of erythrocyte lifespan in small mammals, c) temperature dependence of physiological rate effects, d) relations between brain weight, body size, energy metabolism, and longevity.

#### B. Dynamics of Self-Renewing Cell Populations

This study attempts to find appropriate mathematical formalisms describing the behavior of cellular populations. Because the detailed laws governing birth, death, and transformation into other cell types are largely unknown, the theory is confined essentially to a statistical and/or a phenomenological treatment of the population properties. The most elaborate form is provided by the mathematical methods of T. E. Harris, J. E. Moyal, and others. A simplified version of the probabilistic theory that is useful in dealing with large populations is based on a differential equation originally introduced by H. Von Foerster and similar to the equation of continuity in hydrodynamics. To solve specific problems, machine calculations are unavoidable; use of electronic computers will become more and more important as this work progresses.

#### C. Statistical Description of Acute Radiation Injury Processes

This program investigates the mechanisms of acute lethal radiation injury in higher vertebrates. Emphasis is upon the identification and separation of mechanisms involved in acute death and on the description of the time-dose relations for processes of injury accumulation and for their repair. Acute lethal effects are assessed by the mortality that occurs within 30 days after ionizing irradiation, and are considered in relation to the symptom complexes and the pathology that accompany them. The objectives include: (1) a description of the dose-time relations for mortality attributed to identifiable modes of death; (2) the determinations of alterations in mortality patterns between protracted and fractionated radiation exposures; and (3) the assessment of differences due to radiation quality. Radiation conditions involve protracted, split, or fractionated exposures administered singly and in combination.



## D. Comparative Physiology and Psychology of Longevity and Senescence

Interspecies prediction of life-shortening actions of radiation depends on knowing the different factors that govern species longevity, and determining the radiosensitivity for each factor separately. Following a lead given by the previously developed stochastic theory of mortality, data were collected which proved that longevity of most mammalian species including man can be accurately predicted by the two factors of body weight and brain weight.

On the basis of the results of a statistical analysis of brain weight-body weight-life span relations in rodents, insectivores and small primates, it was decided to investigate the dependence of longevity, aging rate, and radiosensitivity on brain and body size within Rodentia, by comparing species pairs such as Mus musculus with Peromyscus, Mastomys with Tamias, etc. In each pair, the members are roughly equal in body weight but differ by a factor of 2 in brain weight. Body size varies by a factor of 5, longevity by a factor of 3.

At present, 6 species have been selected for intensive investigation. Individuals of young adult age will be used in longitudinal studies. A battery of physiological, biochemical and behavioral tests is being developed to answer such questions as: How does rate of aging depend on metabolic rate and brain size, each independent of the other? Do longer lived, larger-brained animals have better physiological regulations? Do they have greater adaptability to environmental changes? Do they have superior immune competences? Do they have superior abilities for psychophysical discrimination, learning, remembering, etc.?

W-7401-ENG-49

Robert C. Baster

Atomic Energy Project

University of Rochester

Rochester, New York

### Radiobiologic Studies with Drosophila

Although the fruit fly, *Drosophila Melanogaster*, has been used extensively in genetic studies neither it nor other insects have been examined at all completely with respect to the response of the adult to radiation. Apart from providing a source of knowledge of the radiobiology of the insect, the fruit fly possesses certain advantages for the study of radiation effects in general; it is easily reared; its mean life span is short, about 50 days; with adequate care it appears to be relatively free of disease in that its survival time is consistent; and its cells are post-mitotic. Therefore, a thorough study of its response to radiation may be made with relative economy and the results may be of considerable value



in efficient planning of studies on longer lived organisms as well as in their own right. Currently, studies of both the acute lethal dose versus age and the life-span versus dose relationships in the adult fly are well underway both as to observation and analysis. When these are complete the carry over of injury from earlier stages to the adult will be investigated.

W-7401-ENG-49  
George Casarett and Hugh Eddy  
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### Pathologic Mechanisms of Permanent and Delayed Radiation Effects

Because the aims of this work are so broad and far reaching, and the approaches and methodology are multidisciplinary, there are, as may well be expected, many specific research projects in the world which relate or touch upon one or another or several aspects of the present project. However, there seems to be no project involving a systematic and thorough multidisciplinary approach to discovering, coordinating and correlating definitively the fundamental morphologic (cytologic, histologic), biochemical, and physiological sequences and mechanisms of irreversible radiation effects in all tissue components of all organs throughout life, in an attempt to relate these to aging processes, mechanisms of disease development (including cancer), and life span.

The ultimate achievement of a highly reliable, practical science of radiological safety and of means to modify or prevent or reverse residual effects of irradiation, will depend greatly on the definitive elucidation of the fundamental pathogenesis and pathophysiologic mechanisms of permanent and delayed radiation effects, such as life span shortening, premature aging, induction and temporal advancement of neoplastic and degenerative diseases, and the variety of degenerative phenomena associated with and preceding these effects. Since radiation is an excellent tool for the study of the general fundamental biologic processes involved, the process of elucidation of the mechanisms of these radiation effects will result in the solution of general problems of aging and disease development, particularly degenerative and neoplastic disease.

The general aims and purposes of this project are to discover systematically the fundamental pathophysiologic nature and mechanisms of the early radiation injuries which are unreversed, how these effects lead in time to the permanent and delayed damage, lesions, and diseases produced, and the nature and extent of the contribution of each organ to the injury, damage and recovery processes in other organs and the body as a whole following irradiation in various modalities (sites, doses, dose rates). Of equal importance is the expected elucidation

of mechanisms of aging and of development of degenerative and neoplastic diseases, our ignorance of which is perhaps the greatest obstacle to a fuller understanding of late radiation effects.

W-7401-ENG-49

George Casarett and Hugh Eddy  
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### Effects of X-Irradiation on Spermatogenesis in Dogs

Although there are several experimental projects in the world involving short or long term studies of effects of ionizing radiations on various aspects of male or female reproductive systems, there seems to be no other project like the present one in which there is study of the life-long effects of brief, briefly fractionated, or life-long irradiation from external sources on spermatogenesis in long-lived mammals, including dose rates near the maximal permissible rates for man.

The information needed for the evaluation of human radiation hazards and the establishment and testing of safety margins in maximal permissible exposure levels is difficult to obtain wholly from the fragmentary observation of man, especially without the insight that can be provided by experimental information. The experimental information most needed for these purposes includes the effects of life-long as well as briefer radiation exposures on the most radiosensitive systems, especially in mammals of similar radiosensitivity to man and having sufficiently long life span as to permit the accumulation of substantial total doses at such low dose rates as those near permissible rates for man.

The chief aims of this project are to provide an appropriate experimental evaluation of the safety margins in dose rates near the range of maximal permissible exposure rates for x- and  $\gamma$ -irradiation set forth by the National Committee on Radiological Protection, by investigating the effects of life-long x-ray exposure of dogs on the highly radiosensitive process of spermatogenesis, and to discover the influence of size of accumulated dose and of dose rate on the production of injury of this system and recover therefrom. Other parameters and systems are also being studied, including hematology, clinical evaluation, gerontologic manifestations, life span and terminal pathology and extensive histopathology.

W-7405-ENG-26  
Takashi Makinodan  
Oak Ridge National Laboratory  
Oak Ridge, Tennessee

#### Growth and Senescence of the Immune Mechanism

Although the immune mechanism of an individual is presumed to play an essential role in aging, very little systematic data have been available on the ability of an individual to respond to an antigen as a function of age. Studies were therefore carried out to determine the primary and secondary antibody-forming potential of mice of varying age groups and of the spleens of donor mice from varying age groups. The purpose of the latter type of study was to establish whether the change in the immune system of an individual with age is due to a change in the functional lymphoid cells or to homeostatic factors regulating the expression of the functional lymphoid tissue. The most significant finding in our first series, which required 6 years of our effort, was that the growth and senescence of the primary antibody-forming potential of intact mice and their spleen are due mainly to increase and decrease, respectively, in the number of progenitor cells. To our knowledge this is the first time senescence of proliferating somatic tissues has been related to undifferentiated progenitor cells rather than to fully differentiated functional cells. Studies are in progress to determine (a) growth and senescence of the secondary antibody-forming potential of intact mice and their spleen as affected by the dose of priming and age of priming, (b) the influence of neonatal and senescent environments on the expression of immune competence of lymphoid cells from donors of varying age groups, and (c) the effect of alteration of the immune competence on the lifespan.

W-7405-ENG-26  
Arthur Upton  
Oak Ridge National Laboratory  
Oak Ridge, Tennessee

#### Long-Term Effects of Irradiation

The purpose is to refine and extend existing data on the physiological and pathological effects of low-level irradiation, with particular reference to late somatic effects. Inbred, micro-biologically defined (caesarian derived, barrier sustained) mice will be exposed to whole-body Cs-137 gamma radiation at various dose rates and in various doses down to 5 rads. The population samples will be large enough to provide statistically reliable data on the dose-response relationship for major effects down to the lowest dose level studied. Following irradiation, mice will be observed throughout life for effects on longevity and disease incidence (leukemia, other neoplasmas, non-neoplastic degenerative diseases, cataracts, and other age-related changes).

Representative animals also will be removed from the colony at intervals for detailed pathological, bacteriological, virological, hematological, physiological, immunological and biochemical examination. Orientation studies to prepare for this investigation are in progress. Quarters to house the mice under a relatively well controlled environment have been developed and are being refined. A germfree colony of RFM/Un mice has been established and characterized microbiologically. From these animals, an expansion colony has been set up and is being expanded to produce the large numbers of animals needed. The first experimental mice from this colony have been set up in a pilot study for life span observation and are being examined for the incidence of leukemia and other diseases. Methods for collection, storage, retrieval, and analysis of the unusually large amounts of data to be generated by the experiment are being developed. Three Cs-137 gamma ray sources for irradiation of the animals have been installed and are being calibrated.

W-7405-ENG-48

Patricia W. Durbin

Lawrence Radiation Laboratory

Berkeley, California

Growth and Senescence of the Soft Tissues  
and Skeleton in the normally Aging Female Rat

Radiation has been shown to retard growth as well as to accelerate aging. Quantitative data on the growth and atrophy of soft tissues and a description of morphological changes that normally occur during maturation or in old age are essential to the understanding of radiation effects. Accumulation of certain radioisotopes in bone is age dependent. This phenomenon can only be explained if the pattern of skeletal growth and the morphological changes that accompany aging are known.

A systematic examination of the growth and aging of the normal female rat has been undertaken using long-lived, essentially parasite and disease-free COBS animals. Cesarean-Originated, Barrier Sustained (COBS) weanling rats were purchased from Charles River Breeding Laboratories in 12 shipments of 50 or more rats over a three-year period. Some rats were scheduled for routine sacrifice at ages from 30 to 500 days, and others were set aside for lifespan observation. All rats were weighed monthly, examined and palpated for external tumors. Moribund rats or those carrying large mammary tumors were sacrificed. Dead rats were examined for tumors and to determine probable cause of death.

Autopsy records included gross findings and weights of soft tissues and bones. Soft tissue and bone specimens were prepared for routine histology. The carcass was reduced to ash and the mineral content of the skeleton was determined. Bone and soft tissue weights and the incidence of external tumors have been tabulated and are being analyzed. Histologic examinations are complete for 4 groups and the pituitary glands and tumors of all rats have been examined.



BUREAU OF HEALTH SERVICES GRANTS AND CONTRACTS

BUREAU OF HEALTH SERVICES  
RESEARCH GRANTS AND CONTRACTS

<u>GRANT NUMBER</u>	<u>INVESTIGATOR, PROJECT TITLE</u>
CH 23-26	Maurice I. May Hebrew Rehabilitation Center for Aged Roslindale, Massachusetts Converging Community Services on a Home-for-Aged Waiting List
CH 27-23	Robert P. Slattery Catholic Charities of St. Louis St. Louis, Missouri Non-Institutional Care of Aged and/or the Chronically Ill
CH 37-19	Mary H. Little Community Chest and Council of the Cincinnati Area Cincinnati, Ohio Home Health Aide Care for the Chronically Ill and Feeble Aged in a Five-County, Two-State Metropolitan Area
CH 34-48	Paul R. Torrons St. Luke's Hospital New York, New York Morningside Gardens Retirement Health Services Research and Demonstration Project
CH 00249	John Colombatos Columbia University New York, New York Physicians and Medicare: A Study of Attitude Change
CH 00329	Nicholas Babchuk University of Nebraska Lincoln, Nebraska Personal Influence and Participation by Older Adults
CH 00362	Irving A. Fowler Research Foundation of the State University of New York Albany, New York Screening Elderly Nursing Home Patient Problems

CH 00363	Adah D. Parker University of Illinois Urbana, Illinois Study of Recreation on Institutionalized Aged
CH 00384	Terence C. Pihlblad University of Missouri Columbia, Missouri Health and Adjustment of Older People in Small Towns
CH 00385	Margaret Blenkner Benjamin Rose Institute Cleveland, Ohio Home Aide Service and the Aged: A Controlled Study
CH 00386	Arthur H. Richardson Brandeis University Waltham, Massachusetts Medical Care of the Very Aged
CH 00392	Eugene A. Friedmann Kansas State University Manhattan, Kansas Changing Community Patterns: Health Services for Aging
CH 29434	Dallas Johnson Public Affairs Committee, Inc. Washington, D.C. Information Referral and Counseling Services for the Chronically Ill and Aged
CH 29436	Robert Manheimer New York Chapter, The Arthritis Foundation, Inc. New York, New York The Neighborhood Approach to Identifying and Meeting the Health and Related Needs of the Aged
CH 34997	Nina B. Woodside D.C. Department of Public Health Washington, District of Columbia Health Services for the Aged in Garfield Terrace Public Housing Project

CH 59458	<p>Mrs. Helen G. Laue  Welfare Council of  Metropolitan Chicago  Chicago, Illinois  A Home-Delivered Meals Service  for the Chronically Ill and  the Aged in Selected Areas  of a Metropolitan Community  with Central Coordination  and Supporting Services</p>
CH 89488	<p>Irma Deane Pirri  Beth Israel Hospital  and Home Society  Denver, Colorado  Coordinated Day Care, Adult  Foster Home and Volunteer  Services for the Aging</p>
CH 99006	<p>Brahna Trager  San Francisco Homemaker Service  San Francisco, California  A Demonstration of District-Based  Health Department In-Home Service  to the Chronically Ill and Aging  in San Francisco</p>
PH 108-66-189	<p>Donald Irish  Genesee Valley Medical Foundation  Rochester, New York  Regional Utilization and  Medical Review in New York State</p>
PH 108-66-233	<p>Joseph Barbaccia  Tulane University  New Orleans, Louisiana  Planning and Initial Establishment  of a Model Health Maintenance  Program in an Urban Community</p>
PH 108-66-281	<p>E. R. Krumbiegel  City of Milwaukee Health Department  Milwaukee, Wisconsin  Develop and Demonstrate a Model  Adult Health Maintenance Program  in an Urban Community</p>

PH 108-67-39

Lois Crooks  
Educational Testing Service  
Princeton, New Jersey  
The Development of a Simulated  
Exercise to be Used in Conjunction  
with the Medicare Workshops for  
Nursing Home Administrators

PH 108-67-41

Leo Gitman  
Brookdale Hospital Center  
Brooklyn, New York  
Development of a Model Hospital-Based  
Health Maintenance Program for a  
High-Density Low-Income Urban  
Population

PH 108-67-66

Melvin Glasser  
Michigan Health and Social  
Security Institute, Inc.  
Detroit, Michigan  
Assessment of Effects of a Nationwide  
Nursing Home Benefit and the Develop-  
ment of a Research Program

PH 108-67-94

Lois Crooks  
Educational Testing Service  
Princeton, New Jersey  
The Editing of Case Histories  
to be Used in Conjunction with  
Workshops for Administrators of  
Potential Extended Care Facilities

PH 108-67-96

John O. Robben  
Montgomery County Medical Society, Inc.  
Silver Spring, Maryland  
Plan for, Establish, and Support  
Initial Operation of a Community-  
Based Utilization Review Plan

PH 110-67-21

A. Ward  
University of Nebraska  
Lincoln, Nebraska  
Establishment of a Center for Geron-  
tology Studies to Provide Continuing  
Education for Health-Related Pro-  
fessionals and Semi-Professionals



PH 110-67-65	<p>Camille Gravel  Louisiana State Health Department  Baton Rouge, Louisiana  Study to Determine Impact of Federal  Health Legislation on the Operation  and Utilization of Louisiana's State  Hospitals and Related Health Insti-  tutions</p>
PH 110-67-83	<p>Raymond White  Idaho Foundation for Medicine and  Biology, Inc.  Boise, Idaho  Design, Implementation, and Evaluation  of Regional Utilization Review Plan</p>
PH 110-67-100	<p>Sandra C. Howell  Gerontological Society  Clayton, Missouri  Development of a Systematic Approach  to Functional Appraisal of Aging  Individuals</p>
PH 110-67-148	<p>Paul M. Lewis  The Hospital Utilization Project  Pittsburgh, Pennsylvania  Utilization Review for Extended  Care Facilities</p>
PH 110-67-185	<p>James G. Roney  Stanford Research Institute  Menlo Park, California  A Study to Assess the Effects of  Medicare on the Providers of  Service Under Part A, Title XVIII,  P.L. 89-97, in Four Communities</p>
PH 110-67-216	<p>Jack Letcher  Louisiana Department of Hospitals  Baton Rouge, Louisiana  Working Relationship Between a State  Health Agency and State Schools of  Higher Education, Extended Care,  Nursing Homes and Related Facilities,  Hospitals, and Other Related Health  Agencies</p>

PH 110-67-217

William DeFries  
Fresno County Health Department  
Fresno, California  
A Pilot Project Designed to Develop  
and Test A Method of Health  
Counseling for Aging Persons,  
Using a Randomly Selected Group  
of Social Security Applicants  
or Beneficiaries as Recipients  
of this Counseling

PH 110-67-231

John Wright  
University of North Carolina School  
of Public Health  
Chapel Hill, North Carolina  
Demonstrating a Method of Collaborative  
Planning for Training Programs to  
Increase Effectiveness of Medicare

PH 110-67-238

William Mosher  
Research for Health in Erie County  
Buffalo, New York  
Pilot Project Designed to Develop  
and Test a Method of Health  
Counseling for Aging Persons

PH 110-67-239

Catherine DiJioia  
The Holyoke Visiting Nurse Association, Inc.  
Holyoke, Massachusetts  
Provide Assistance, Advice, and Services  
in Carrying Out a Pilot Project  
Designed to Develop and Test a Method  
of Health Counseling

CH 23-26

Maurice I. May  
Hebrew Rehabilitation Center for Aged  
Roslindale, Massachusetts

Converging Community Services on a Home-for-Aged Waiting List

In this project, a home and hospital for the aged, together with allied community agencies, will seek to demonstrate a responsible approach to applicants who face a long wait for admission to the home. The objective is to meet the medical and social needs of these aged individuals pending their eventual admission, or possibly even intercepting their institutionalization through appropriate alternative provisions of services.

A multidisciplinary clinical team will evaluate the status and needs of one-half of the home's waiting list of approximately 550 applicants (the other half will serve as a control group), and will marshal existing community resources to those needs. The team will maintain continuing followup on these demonstration cases, reviewing, planning and implementing. Toward the close of the project period, comparisons will be made between the demonstration and control cases (1) as to their status in terms of met and unmet needs and (2) as to what difference intervention made in the size and composition of the resulting waiting list.

CH 27-23

Robert P. Slattery  
Catholic Charities of St. Louis  
St. Louis, Missouri

Non-Institutional Care of Aged and/or the Chronically Ill

It is proposed to identify and develop a method of providing comprehensive home care services for the aged and/or the chronically ill to permit them to remain in their homes as long as possible or to return to their homes from hospitals or institutions. This requires establishing a project for people with a multiplicity of needs which will change and need continual reassessment.

It is to be neither a hospital nor service-oriented project but one that is community oriented. This coordinated service will be directed towards the prevention of disruption of family life and the physical and emotional trauma this causes the patient and his family, both adults and children.

The scope of this project will be limited to all the people in ten neighborhoods which are widely diversified economically, socially and culturally, and to the patients in one general hospital.

After a medical-social assessment of people's needs and strengths, a coordinated plan will be developed to provide the services needed either by this project or by use of community resources, through medical and nursing services, housekeepers, home visitors and the help of neighbors.

The knowledge and experience gained will be disseminated, locally and nationally, through reports, publications, workshops, institutes, professional schools.

CH 37-19  
Mary H. Little  
Community Chest and Council  
of the Cincinnati Area  
Cincinnati, Ohio

Home Health Aide Care for the Chronically Ill and Feeble Aged  
in a Five-County, Two-State Metropolitan Area

The Home Aide Project is a three-year demonstration of home aide care for chronically ill patients and feeble aged persons to enable the individual to function at his most competent level in his own home as long as possible. Home Aide service is focused upon the personal care of the patient. It is designed to meet their daily living needs and is the kind of personal care usually given by a family member. It may include assistance in going to the bathroom, in and out of wheel chair, special diet preparation, supervision or giving of medication according to medical orders. The proposal has been developed after a survey in a five-county two-state area disclosed a very large unmet need.

The Project has four chief objectives: (1) to learn how to give such service in a large metropolitan area covering five counties in two states, (2) to delineate more clearly and specifically the characteristics of such a service as distinguished from traditional homemaker services where the chief concern is for the care of children, (3) to learn how to relate such service to a protective program for older people, (4) to learn how best to correlate the service with a home medical care program.

CH 34-48  
Paul Torrons  
St. Luke's Hospital  
New York, New York

Morningside Gardens Retirement  
Health Services Research and Demonstration Project

The purpose of the proposed project is three-fold: 1) to determine the need for and the utilization of various health and

health-allied services among a middle-income group of persons over 60 years of age living in a cooperative housing project; 2) to determine the ability of this group to meet the needs for these services by utilizing volunteer workers drawn from the group itself; 3) to determine the feasibility of establishing a pre-paid, self-supporting insurance plan to provide those services which cannot be provided by the volunteer workers.

CH 00249

John Colombotos

Columbia University

New York, New York

### Physicians and Medicare: A Study of Attitude Change

This research study has two aims: (1) to examine the effects of the passage of the Medicare legislation on physicians' attitudes toward Medicare, and (2) to examine two sets of conditions under which physicians' attitudes toward Medicare change:

a) The consistency of other attitudes with attitudes toward Medicare.

It is predicted, for example, that among those physicians initially opposed to Medicare, those who are politically liberal are more likely to develop favorable attitudes toward Medicare than those who are politically conservative.

b) The social context of the physicians.

It is predicted, for example, that physicians in New York City, where half the physicians were in favor of Medicare before its passage, are more likely to maintain and develop favorable attitudes toward Medicare than physicians in upstate New York counties, where only a fifth of the physicians were initially in favor of Medicare.

This study builds on interviews with a probability sample of 1,205 private practitioners in New York State conducted in 1964 and early 1965. The interviews included questions on Medicare, Government medicine, the organization of medical practice, political ideology, and other related issues. It is proposed to re-interview this sample between April 15 and June 30, 1966 -- nearly one year after the passage of Medicare and just before it goes into effect.

The availability of these interview data collected before the passage of Medicare provides a unique opportunity to examine-- in natural, field situation--the effects of legislation and the conditions producing attitude change, problems of central importance in social-psychological theory and research.



CH 00329  
Nicholas Babchuk  
University of Nebraska  
Lincoln, Nebraska

#### Personal Influence and Participation by Older Adults

The principal objective of the proposed study is to acquire information that will enable professional persons concerned with maintaining the physical and mental health of the aged to employ more effective procedures to involve older adults in voluntary associations, adult education programs and certain community health services. Such professional persons would include community organizers, social workers, health and welfare agency directors and adult educators.

The focus of this study is the older adult who is most likely to participate in such organizations -- those having few health constraints who tended to be somewhat active earlier in life rather than the disabled or person having very little or no contact with such formal organizations earlier in life. The objective of this study will be achieved through a comprehensive investigation of the ways in which older adults become involved in organizations. The concept of "personal influence" provides a useful theoretical framework within which to examine decisions made by older adults to participate in age-graded organizations, and to make use of various health services. Major theoretical constructs and research findings related to the concept of personal influence will be applied to and tested relative to the participatory decisions of older adults.

CH 00362  
Irving A. Fowler  
Research Foundation of the  
State University of New York  
Albany, New York

#### Screening Elderly Nursing Home Patient Problems

This research is aimed at a successful demonstration that a "diagnostic instrument," which can identify those patients in nursing homes who have certain social or personal problems, can be developed for use by non-social work nursing home staff members. The investigator's on-going research pursuant to this goal has been sufficiently encouraging to recommend the research proposed: a rigorous testing of the instrument's accuracy of diagnosis. The ultimate research objective lies beyond a successful outcome of this test: a diagnostic tool for use by nursing home staff that will be an

inexpensive, easily available means for pinpointing patients who need social service help. Making such a determination without using scarce social work professional time would be beneficial since that time could then be spent in treating selected patients rather than in screening all patients. Furthermore, the instrument's use by nursing home staff could, to a considerable degree, contribute to their professionalism by increasing their awareness of patient's social and emotional needs.

CH 00363  
Adah D. Parker  
University of Illinois  
Urbana, Illinois

#### Study of Recreation on Institutionalized Aged

Often a home for the aged is regarded as a place where older adults, having served their purpose in life, are placed in isolation to pass their remaining years in apathy. Many people assume that a home for the aged is about the same as a "poor house." Generally, administrators of institutions for the aged are of necessity primarily concerned with the problems related to medical attention and custodial care. Research needs to be undertaken to reveal some specific action which could be taken to enrich the lives of the institutionalized aged and define new areas of study needed to discover further scientific data regarding recreation in the institutionalized setting.

This study will evaluate the hypothesis that a professionally directed recreational program for the aged will influence favorably the following objective indexes: (1) the amount of nursing care, (2) the amount and kinds of medications, (3) frequency of night wanderings, (4) personal grooming, (5) social adjustment, and (6) patient satisfaction and positive involvement.

CH 00384  
Terence C. Pihlblad  
University of Missouri  
Columbia, Missouri

#### Health and Adjustment of Older People in Small Towns

The central objective of this project is a survey and analysis of the health conditions and needs of older persons residing in small towns of Missouri and of the relation of health to social participation and personal and social adjustment of older people in the small town.

There is a scarcity of information concerning the problems of aging in the small community. Most of the research in this field has been concentrated in urban areas and relatively little is known concerning the status of old people in the smaller community. It seems probable that some of the generalizations concerning the needs and the social organization to meet the needs of older people in an urban setting are of limited applicability to the rural community and to the aged in the small town.

This is planned as a field study and will accumulate 2000 interviews of older persons in their homes. The study sites will be twelve towns representing different population size categories and contrasting social areas of the state.

CH 00385

Margaret Blenkner

Benjamin Rose Institute

Cleveland, Ohio

#### Home Aid Service and the Aged: A Controlled Study

This four-year study will examine the effect of home aide service upon aged persons. Home aide service is defined as those personal care and management functions normally performed by an individual himself, or by a family member, and in which the major concern is daily maintenance, comfort, and support.

A defined aged population (dischargees from a geriatric rehabilitation hospital) will be eligible for participation in the study provided they meet the following criteria: (a) must be 60 years of age or older; (b) must not absolutely require intensive nursing or custodial care; (c) must have a non-institutional place of abode. Those selected will be randomly divided into an experimental group, which will be eligible for home aide service for a period of two years, and a control group, which will not be eligible for such service.

Several null hypotheses will be tested in order to determine whether the fate of the participants is positively and significantly changed by providing them with home aide service. Fate refers to survival (measured in terms of mortality and longevity), life satisfaction (measured in terms of a contentment scale), and institutionalization (measured in terms of relocation to an institution from a non-institutional place of abode).

CH 00386

Arthur H. Richardson  
Brandeis University  
Waltham, Massachusetts

Medical Care of the Very Aged

The investigation will provide information on the use of health resources by a group of males 80 years of age and over while covered by a moderate third-party payment health plan and then will describe the changes that occur in use of medical resources after the implementation of a comprehensive medical care program. The study is directed at specifying social and medical characteristics associated with patterns of health-resource use and at identifying variables associated with the modification of these patterns under an expanded health-benefit program.

The study will take into account the associations between prior medical experience, perceived medical needs, style of life measures, and structural-ecological factors to utilization and changes in utilization of health resources. Data will be obtained by means of a panel-interview study, supplemented by information from health insurance and hospital records. Some 1500 former male employees of the "big-three" automobile manufacturers constitute the study group.

This study will provide basic data on the medical needs and treatment behavior of the very aged, and will assess the impact of federal and union health programs on health-resource use. Findings will have implications for the planning of health resources for the very aged and the estimation of medical care resources required under a broad third-party payment health program.

CH 00392

Eugene A. Friedmann  
Kansas State University  
Manhattan, Kansas

Changing Community Patterns: Health Services for Aging

This project will study the impact on medical care of the aged of the Social Security Amendments of 1965 in the midwest region of the United States. The investigators propose to determine the relationship between increased ability to pay for health care services and (1) utilization by the individual, (2) the provision of services by the community, and (3) the changing relationships between utilization and provision. These variables will be examined over a two-year period in a variety of communities which differ in size and other characteristics.

CH 29434  
Dallas Johnson  
Public Affairs Committee, Inc.  
Washington, D.C.

Information Referral and Counseling Services for  
Chronically Ill and Aged

This project provides for the production and utilization of a documentary film, a public affairs pamphlet, and program discussion guides designed to encourage community groups to develop and strengthen information, referral and counseling services for the chronically ill, and the aged.

The Film will be from 24 to 29 minutes in length, designed to motivate its viewers into an action program. The pamphlet will carry the background information necessary for such a project. The discussion and program guide will seek to bridge the gap between the mythical "Anywhere, U.S.A." community portrayed in the film, to the community in which the film is being shown. It will attempt to provide group leaders with guidance in encouraging discussion and an action program at home. The demonstration and evaluation portion of the program will focus on two states -- one predominately urban and the other predominately rural, and on two cities -- one a metropolis, and the other a medium-sized city. Varying combinations of the film, the pamphlet, and the discussion guide will be used and then effectiveness will be measured. Two national organizations, the National Health Council and the United Community Funds and Councils of America will cooperate in the demonstration phase of the program.

CH 29436  
Robert Manheimer  
New York Chapter, The Arthritis  
Foundation, Inc.  
New York, New York

The Neighborhood Approach to Identifying and  
Meeting the Health and Related Needs of the Aged

The objectives of this project are as follows: 1) Demonstrate the function of a neighborhood "middleman" service, which would identify elderly persons with health and related problems and would help them utilize all of the resources of the community to meet those needs. 2) Develop through use precise case finding techniques for reaching all the aged in an area and for becoming identified as a helping service. 3) Learn from case experience how existing health and welfare agencies can be made to serve the



needs of the infirm aged. 4) Identify and analyze "gaps" in service -- whether they reflect low utilization, or the need for new methods for delivery of services, or development of additional types of services. 5) Organize community support to influence both community and agency service structure. 6) Demonstrate the use of nonprofessionals in case finding and case aide roles, as a means of further extending the reach of the professional worker. 7) Show how nonprofessionals can be trained for this work, and whether they can, over several years, maintain their interest in this type of program. 8) Explore sources of sponsorship for continuing and replicating the program, and develop replicated models.

CH 34997

Nina B. Woodside

D.C. Department of Public Health

Washington, District of Columbia

Health Services for the Aged  
in Garfield Terrace Public Housing Project

Garfield Terrace is a low income public housing development in an economically deprived area of Washington, D.C. One building is for occupancy exclusively by an estimated 342 aged persons. Another nearby public housing project plus adjacent census tracts will yield a total of almost 3,000 aged to be served by the third year of this project. The National Capital Housing Authority has made available to the D.C. Health Department 2,000 square feet of space for a health facility on the main floor of the building housing aged residents. The space is completed to our specifications and is now ready for occupancy.

A team of medical and paramedical personnel will provide coordinated service for the aged residents, in their own apartment as well as in the health facility. Services will include those of an internist, public health nurse, licensed practical nurse, physical therapist, nutritionist, home economist, and social worker.

By providing for the aged residents of Garfield Terrace an "on the spot" facility for routine periodic health maintenance as well as for detection of unknown chronic diseases, and by providing for coordinated care to restore them to maximum physical and mental function, these aged will be able to maintain an independent existence at home to a greater extent than is possible if they must travel any distance to a variety of facilities for fragmented services.

CH 59458  
Helen G. Laue  
Welfare Council of  
Metropolitan Chicago  
Chicago, Illinois

A Home Delivered Meals Service for the Chronically  
Ill and Aged in Selected Areas of a Metropolitan Community  
with Central Coordination and Supporting Services

The project proposes to: 1) Demonstrate a coordinated metropolitan plan for providing home delivered meals five days a week to the chronically ill and the aged in selected areas of a metropolitan community in which substantial need for such services is indicated; 2) Develop methods of operation, standards of service and patterns of cooperation to enable the service to continue and expand with a minimum of coordination; 3) Develop, demonstrate and prepare teaching materials on nutrition counseling to help clients use well the food served and plan for their other meals; and 4) Document and evaluate administration, services, recipients and needs.

The Welfare Council will coordinate the program and provide certain central services. Catholic Charities, Hull House, Lutheran Social Service and Olivet Community Center will be responsible for the service to clients and for recruiting and supervising volunteers to deliver the meals.

Public health nursing agencies will provide nursing evaluation of the clients who must be under a doctor's supervision at least for positive health guidance on an annual basis. The Visiting Nurse Association will supervise a nutritionist who will develop and test educational procedures required in nutrition counseling. The Chicago Dietetic Association will provide consultation service.

CH 89488  
Irma Deane Pirri  
Beth Israel Hospital and Home Society  
Denver, Colorado

Coordinated Day Care Adult Foster Home  
and Volunteer Services for the Aging

Sponsored by the Beth Israel Hospital and Home Society complex of comprehensive acute, chronic, and residential care, this project proposes to demonstrate that aging individuals can be maintained within the community on an effective functional level by developing and administering an integrated program of medical, social, and personal services in their behalf.

Methods employed will be: (1) to develop an intake procedure sufficiently extensive to involve the client, his family, and physician in diagnosis and evaluation of the older adult's immediate and long-term physiosocial needs, (2) to provide structured Day Care services within the Beth Israel Home for the Aged for both in-and-out of hospital patient-clients, (3) to maintain the personal autonomy of the aging through full utilization of approved Adult Foster Homes, (4) to enhance and strengthen services to the elderly by the use of a carefully selected, trained and supervised Volunteer Service Corps, (5) to evaluate the role of the volunteer as a therapeutic team member, (6) to stimulate inter-agency cooperation in the use of each other's facilities and services, and (7) to design procedures to assess the outcomes of the project and gauge its effectiveness through measuring the quality and extent of services given.

It is anticipated that the successful demonstration of this project will motivate the agencies and groups concerned with the geriatric person to sponsor and support a central agency to coordinate the existing fragmented services and provide the needed additional services for the elderly in metropolitan Denver.

CH 99006

Brahna Trager

San Francisco Homemaker Service

San Francisco, California

A Demonstration of District-Based Health Department In-Home  
Service to the Chronically Ill and Aging in  
San Francisco

The project is intended to determine the extent of Health Department potentials in a program for the chronically ill and aging which demonstrates the most effective methods of using Public Health philosophy, staff and skills.

Primary emphasis will be placed upon determining whether the Health Department should become a major community resource and the extent to which the District Health Center should provide: (1) Evaluation of total individual patient needs and planning of services adapted to those needs through the use of qualified personnel. 2) Information and referral to appropriate resources; counselling and related social services. 3) Continuous surveillance and coordination; make necessary service plan changes and offer support through the staff of the district health center in order to assist patients to achieve optimum health and make the best possible use of services. 4) Direct in-home services in order to support such plans. These will include homemaker-home health aide services. In addition other related in-home services will be secured as required.

PH 108-66-189  
Donald Irish  
Genesee Valley Medical Foundation  
Rochester, New York

Regional Utilization and Medical Review in New York State

The Contractor will plan and implement a Regional Utilization Review Plan in the 11 counties that comprise the Rochester Regional Hospital Council and the Seventh District of the Medical Society of the State of New York. The plan will include a broadly representative central coordination committee and subcommittees concerned with utilization review procedures in hospitals and extended care facilities and with insurance claim procedures. The plan shall be an integrated regional approach to the utilization of review procedures, in hospitals and extended care facilities and with insurance claim procedures under Parts A and B of the Health Insurance for the Aged Program.

PH 108-66-233  
Joseph Barbaccia  
Tulane University  
New Orleans, Louisiana

Planning and Initial Establishment of a  
Model Health Maintenance Program in an Urban Community

The Contractor will develop and establish a Model Adult Health Maintenance Program in an urban community using, to the extent feasible, automated equipment and techniques which will achieve optimum utilization of the time of physicians and other health workers and which will permit the health assessment of large numbers of adults in an economical and effective manner. The program will include health information and motivation, periodic health assessment, and counseling, referral, and follow-up.

PH 108-66-281  
E. R. Krumbiegel  
City of Milwaukee Health Department  
Milwaukee, Wisconsin

Develop and Demonstrate a Model Adult Health Maintenance Program  
in an Urban Community

The Contractor will develop and demonstrate a model adult health maintenance program in an urban community using, to the extent feasible, automated equipment and techniques to achieve optimum utilization of the time of physicians and other health workers, and to permit the health assessment of large numbers of adults in an economical and effective way.



PH 108-67-39  
Lois Crooks  
Educational Testing Service  
Princeton, New Jersey

The Development of a Simulated Exercise to be Used in Conjunction  
with the Medicare Workshops for Nursing Home Administrators

The Contractor shall, based upon a body of administrative problems relating to nursing homes administration furnished by the Public Health Service (with the collaboration of the American College of Nursing Home Administrators), develop two simulated in-basket exercises for use during the Rutgers University Home Administrators Workshop (August 16-18, 1966), observe the two administrations, and/or consult on revisions of material for use in the future.

PH 108-67-41  
Leo Gitman  
Brookdale Hospital Center  
Brooklyn, New York

Development of a Model Hospital-Based Health  
Maintenance Program for a High-Density Low-Income Urban Population

The Contractor will develop and demonstrate a model adult health maintenance program in an urban community using, to the extent feasible, automated equipment and techniques to achieve optimum utilization of the time of physicians and other health workers, and to permit the health assessment of large numbers of adults in an economical and effective way.

PH 108-67-66  
Melvin Glasser  
Michigan Health and  
Social Security Institute, Inc.  
Detroit, Michigan

Assessment of Effects of a Nationwide Nursing Home Benefit and  
the Development of a Research Program

The Contractor will establish a reporting network which will forward to the Institute information on the operations of the various components of the nursing home benefits including providers of care, insurance carriers, and beneficiaries. The Contractor will summarize all pertinent information and forward reports to the Project Officer monthly. The activities will be directed toward the exploration of the impact of the whole program of nursing home benefits on beneficiaries, providers of service and insurance carriers to develop a series of research proposals. These research proposals will have as their central focus the basic questions of "What are the components of a successful nursing home benefit?" and "How are these organized to supply care that is appropriate and economical?"



PH 108-67-94  
Lois Crooks  
Educational Testing Service  
Princeton, New Jersey

The Editing of Case Histories to be Used  
in Conjunction with Workshops for  
Administrators of Potential Extended Care Facilities

The Contractor is to revise, edit and supplement the following teaching resource materials developed for instructional use: two In-Basket Exercises; three case studies in Utilization Review; three case studies in Patient Care Policy; and one case study each in the areas of Transfer Agreements, Reimbursement Principles, Physical Therapy, Medical Social Service, Nutritional Services, Occupational Therapy, Pharmacy, Dental Services, Podiatry, Speech Therapy, and Medical Records.

PH 108-67-96  
John O. Robben  
Montgomery County Medical Society, Inc.  
Silver Spring, Maryland

Plan for, Establish, and Support Initial Operation of  
a Community-Based Utilization Review Plan

The Contractor will plan for, establish, and support initial operation of a community-based utilization review plan which shall include two or more extended care facilities and any interested hospitals in the community. The plan will be in conformity with the provisions for utilization review established in the Conditions of Participation for Hospitals and for Extended Care Facilities. He will provide staff and assistance to interested extended care facilities and hospitals in the community to plan and implement utilization review procedures. He will develop a utilization review plan.

PH 110-67-21  
A. Ward  
University of Nebraska  
Lincoln, Nebraska

Establishment of a Center for Gerontology Studies to  
Provide Continuing Education for Health-Related Professionals  
and Semi-Professionals

The objectives of the project are to (1) develop a mechanism (the Center) through which short courses in gerontology may be offered

to practicing health and health-related personnel and (2) develop and present challenging programs in gerontology for these groups. A plan will be submitted which shall include specific objectives for a second year of operation and the methodology for accomplishing these objectives.

PH 110-67-65  
Camille Gravel  
Louisiana State Health Department  
Baton Rouge, Louisiana

Study to Determine Impact of Federal Health Legislation  
on the Operation and Utilization  
of Louisiana's State Hospitals and Related Health Institutions

The Contractor will: (1) develop a system and methodology for studying the impact of existing Federal Health legislation on the operation and utilization of Louisiana's State Hospitals and related health institutions and facilities; (2) conduct the study in accordance with the system and methodology developed; and (3) analyze the study findings and develop predictive models showing the future role of such hospitals, institutions, and facilities.

Federal Health legislation enacted subsequent to the beginning of the contract period will be included in the study.

PH 110-67-83  
Raymond White  
Idaho Foundation for  
Medicine and Biology, Inc.  
Boise, Idaho

Design, Implementation, and Evaluation  
of Regional Utilization Review Plan

The Contractor will design, implement, and evaluate a regional utilization review plan for one of three major regions of the State of Idaho -- the Treasure Valley Area. The design will provide for a broadly representative central coordinating committee, including professional and public representatives from the region being served and of the facilities and agencies involved, and fiscal intermediaries for the Health Insurance program. The program will be an integrated regional approach to utilization review and will meet requirements under the Health Insurance for the Aged program.

PH 110-67-100  
Sandra C. Howell  
Gerontological Society  
Clayton, Missouri

Development of a Systematic Approach to  
Functional Appraisal of Aging Individuals

The contractor will develop a systematic approach to the functional i.e., physical, social, and intellectual appraisal of the aging (over 40) individual, suitable for intramural testing by health practitioners in PHS hospitals. The aim is to produce a tool for use in the prevention and containment of disability in middle and older age groups.

PH 110-67-148  
Paul M. Lewis  
The Hospital Utilization Project  
Pittsburgh, Pennsylvania

Utilization Review for Extended Care Facilities

The Contractor will design, initiate, conduct, and evaluate a demonstration and study of utilization review in at least eight extended care facilities in western Pennsylvania. The total bed capacity of the facilities participating in the program will be a minimum of 2,800 beds.

PH 110-67-185  
James G. Roney  
Stanford Research Institute  
Menlo Park, California

A Study to Assess the Effects of Medicare on  
the Providers of Service Under Part A,  
Title XVIII, P.L. 89-97, in Four Communities

The Contractor will develop a methodology to carry out the necessary compilation and analysis of existing data in the four communities and to gather comparable data for the post-Medicare stage. He will then analyze the before and after Medicare data to show the changes which have occurred in the patterns of use of health care facilities. This study, combined with the results of others now in progress, will serve as a basis for estimating the future demands for health services among the 65 and older populations.

PH 110-67-216  
Jack Letcher  
Louisiana Department of Hospitals  
Baton Rouge, Louisiana

Working Relationship Between a State Health Agency  
and State Schools of Higher Education, Extended Care,  
Nursing Homes and Related Facilities, Hospitals,  
and Other Related Health Agencies

The purpose of this contract is to develop a working relationship between the official State Health Agency responsible for licensure and Medicare programs and State schools of higher education, extended care facilities, nursing homes and related facilities, hospitals, and other related agencies. The quality and quantity of care and services to patients will thus be increased through effective utilization of available professional medical and paramedical personnel and bringing the nursing home into the mainstream of community health resources.

PH 110-67-217  
William De Fries  
Fresno County Health Department  
Fresno, California

A Pilot Project Designed to Develop and Test a Method  
of Health Counseling for Aging Persons, Using a Randomly Selected  
Group of Social Security Applicants or Beneficiaries  
as Recipients of this Counseling

The Contractor will conduct a pilot project designed to develop and test a method of Health Counseling for Aging Persons in Fresno County. The objective will be to provide health counseling services for Social Security applicants, or other aging population, which motivate adoption of a personal program of health protection. (One activity in a project being conducted pursuant to an agreement between PHS and SSA).

PH 110-67-231  
John Wright  
University of North Carolina  
School of Public Health  
Chapel Hill, North Carolina

Demonstrating a Method of Collaborative Planning for  
Training Programs to Increase Effectiveness of Medicare

The purpose of this contract is to demonstrate a method of collaborative planning for training programs to increase

effectiveness of Medicare in Georgia. The Contractor will establish an advisory committee representing providers of service and resources for training within the State. The primary function of this committee will be to assist a training coordinator in identifying training needs which are known to exist within Georgia. The identification of these needs will assist hospitals, extended care facilities and home health agencies in overcoming deficiencies disclosed during the certification of these institutions for participation in Medicare, and will encourage planning and coordination of services for all provider groups. The coordinator will then stimulate and assist identified training resources to develop and direct at least two training programs.

PH 110-67-238  
William Mosher  
Research for Health in Erie County  
Buffalo, New York

Pilot Project Designed to Develop and Test  
a Method of Health Counseling for Aging Persons

The Contractor will conduct a pilot project designed to develop and test a method of Health Counseling for Aging Persons. The objective will be to provide health counseling services for Social Security applicants, or other aging population, which motivate adoption of a personal program of health protection. (One activity in a project being conducted pursuant to an agreement between PHS and SSA.)

PH 110-67-239  
Catherine DiJioia  
The Holyoke Visiting Nurse Association, Inc.  
Holyoke, Massachusetts

Provide Assistance, Advice, and Services in Carrying Out  
a Pilot Project Designed to Develop and Test a  
Method of Health Counseling

The purpose of this contract is to provide assistance, advice, and services in carrying out a pilot project designed to develop and test a method of health counseling for the aged, using a selected group of Social Security Applicants or beneficiaries as recipients of this counseling. The health counseling will include recommendations of positive health measures (especially regular health appraisal) and appropriate referral to community, public, and private health and health-related resources.





